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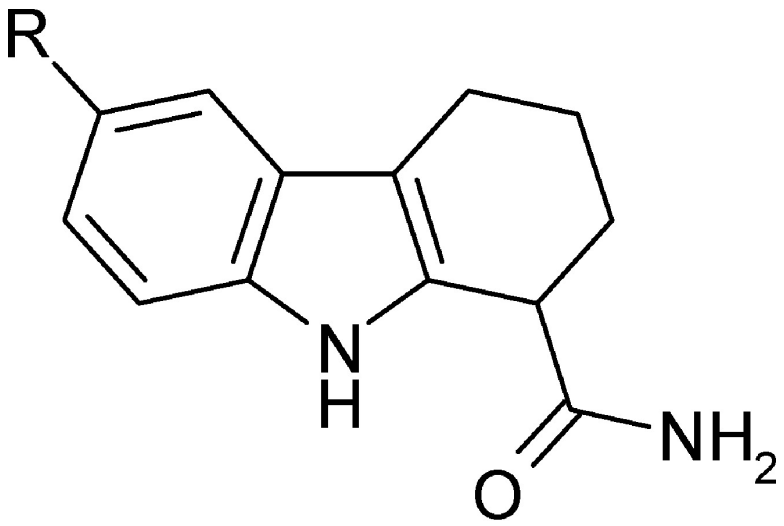

1: R = Cl
2: R = CH_3
3: R = H

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Discovery of Indoles as Potent and Selective Inhibitors of the Deacetylase SIRT1

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High-throughput screening against the human sirtuin SIRT1 led to the discovery of a series of indoles as potent inhibitors that are selective for SIRT1 over other deacetylases and NAD-processing enzymes. The most potent compounds described herein inhibit SIRT1 with IC_{50} values of 60−100 nM, representing a 500-fold improvement over previously reported SIRT inhibitors. Preparation of enantiomerically pure indole derivatives allowed for their characterization in vitro and in vivo. Kinetic analyses suggest that these inhibitors bind after the release of nicotinamide from the enzyme and prevent the release of deacetylated peptide and *O*-acetyl-ADP-ribose, the products of enzyme-catalyzed deacetylation. These SIRT1 inhibitors are low molecular weight, cell-permeable, orally bioavailable, and metabolically stable. These compounds provide chemical tools to study the biology of SIRT1 and to explore therapeutic uses for SIRT1 inhibitors.

Introduction

SIRT1 is a member of the sirtuin deacetylase family of enzymes that removes acetyl groups from lysine residues in histones and other proteins. Sirtuins are the mammalian orthologues of the yeast Sir2 protein, which is implicated in chromatin silencing and lifespan regulation.[1] Although there are seven mammalian sirtuins (SIRT1−7), deacetylase activity and substrates have only been reported for SIRT1, -2, -3 and -5.[1−3] SIRT1 has been implicated in the regulation of fat mobilization, muscle development, axonal degeneration, and cell death.[4−7] Several transcription factors are deacetylated by SIRT1, including members of the FoxO family,[7−10] HES-1 and HEY-2,[11] PPARγ,[4] MyoD,[5] CTIP2,[12] and NF-κB.[13] SIRT1 has also been shown to deacetylate the p53 tumor suppressor protein,[14−16] a key transcriptional regulator of genes involved in cell cycle regulation, apoptosis, and DNA repair.

The SIRTs and yeast Sir2 are unusual deacetylases in that they are dependent on NAD for activity.[17,18] Enzyme-catalyzed protein deacetylation requires the cleavage of the glycosidic bond between the ADP-ribose and nicotinamide moieties of NAD, with concomitant release of nicotinamide. This is followed by transfer of the acetyl group from lysine to form *O*-acetyl-ADP-ribose, as shown in Figure 1.[19−21] The NAD cleavage step is reversible in that nicotinamide can rebind to the enzyme and regenerate NAD, termed nicotinamide exchange. As nicotinamide accumulates during enzymatic turnover, nicotinamide exchange is increasingly favored over acetyl transfer, resulting in product inhibition.[21−23] This SIRT reaction mechanism is fundamentally different from that used by class I and class II HDACs, which remove acetyl groups through zinc-mediated hydrolysis.[24] SIRTs do not possess an active-site zinc; accordingly, their catalytic activity is not affected by the hydroxamate-based HDAC inhibitors such as trichostatin A.[15,17]

To understand better the biology of SIRT1 and to generate potential therapeutics, we sought to identify potent and selective inhibitors of this enzyme. To date, the reported inhibitors are of low potency and solubility, rendering them poor starting points for lead discovery.[25−28] Thus we performed a high-throughput screen against recombinant human SIRT1 using a fluorimetric assay,[29] from which we identified a highly promising series of indoles, shown in Figure 2. Herein we describe the synthesis and profiling of a series of analogues, the most potent of which were assessed for selectivity, ADME properties, and mechanism of inhibition.

Chemistry

Several indoles, shown in Figure 2, were identified as inhibitors of SIRT1 in a high-throughput screen. The most potent of these, compound **1**, was synthesized by two routes, the first of which is shown in Scheme 1. Tetrahydrocarbazole **5** was obtained by cyclization of bromo ketone **4** and *p*-chloroaniline. Hydrolysis of this ester and condensation of the resulting acid **8** with *S*-α-methylbenzylamine gave amide **10** as a mixture of diastereomers. Acid hydrolysis of **10** gave racemic **1**. The two diastereomers of **10** were separable by chromatography, but racemization during subsequent acid hydrolysis prevented isolation of the two enantiomers of **1** by this route. The second route to **1**, also used to prepare analogue **32**, consisted of direct conversion of

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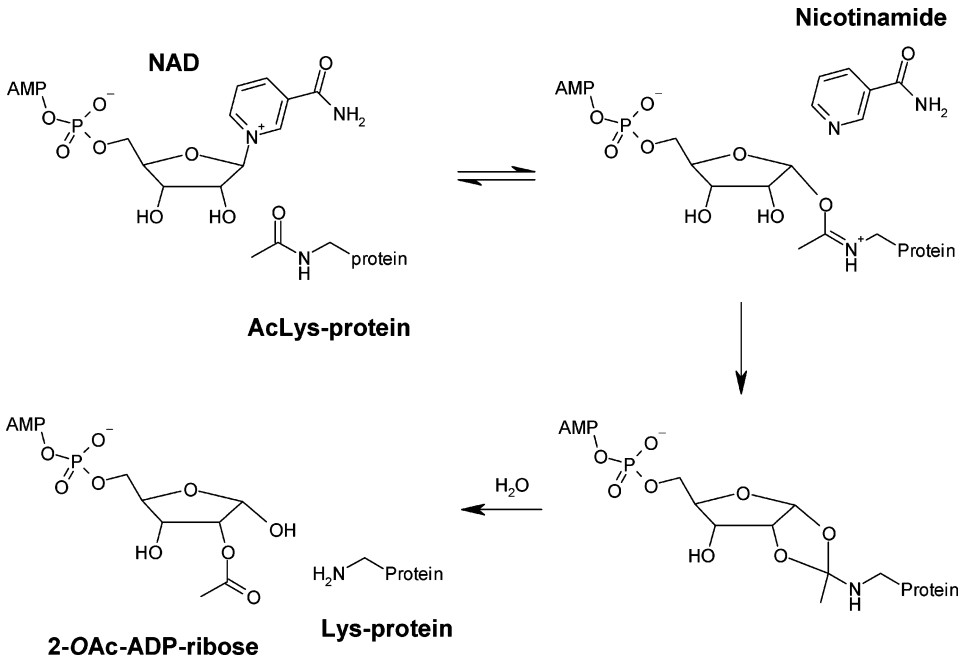


Figure 1. Mechanism of SIRT-catalyzed deacetylation.

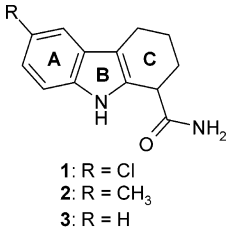


Figure 2. Lead series identified in high-throughput screen.

the ester to the carboxamide by reaction with ammonia under pressure, as shown in Scheme 4. This reaction was readily scaled to 10 g, and subsequent preparative chiral HPLC gave multigram quantities of the enantiomers of **1**.

Syntheses of β-carboline analogues of compound **1** are shown in Scheme 2. Base-catalyzed condensation and cyclization of glyoxylic acid with the indoles **16** and **17** gave the β-carbolines **18** and **19**, respectively. Treatment with ammonia in the presence of the coupling agent PyAOP gave the corresponding amides **20** and **21**. The acetylated derivative **23** was prepared by reaction of amine **18** with acetic anhydride to give acetamide **22**, followed by coupling of **22** with ammonia mediated by HOBt and EDC.

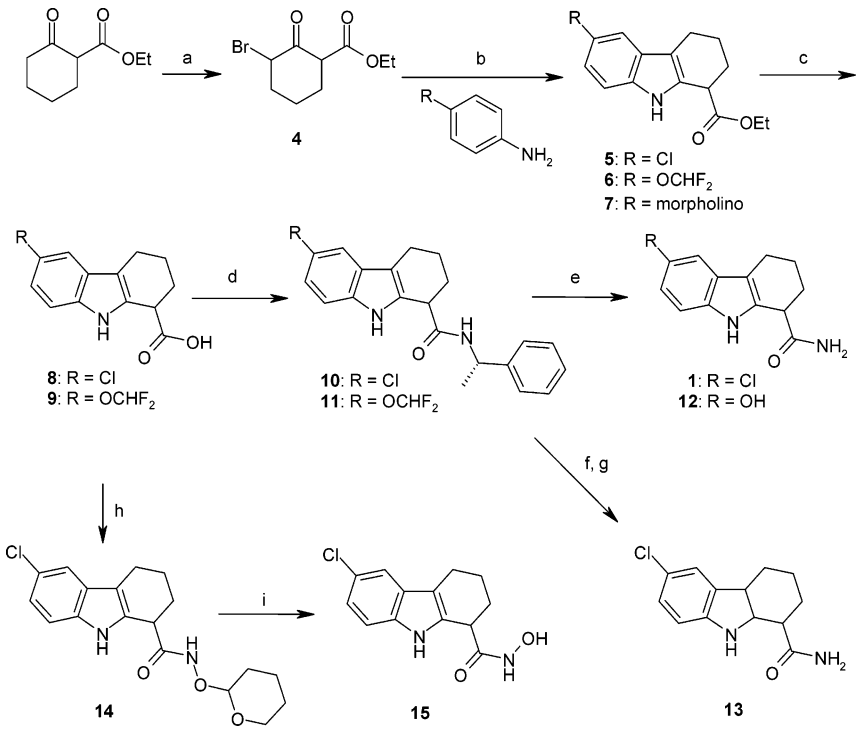
Other analogues incorporating changes in the size and substitution pattern of the C-ring (Figure 2) were synthesized as follows: Compound **13**, the analogue of **1** containing a fully saturated C-ring, was obtained from **10** via borane reduction (Scheme 1). Variants of **1** with the carboxamide group in the 2-position were synthesized as shown in Scheme 3. Fisher indole synthesis gave the carboxylic acids **24** and **25**; these were condensed with *S*-α-methylbenzylamine, and the resulting benzyl amides were subjected to acid hydrolysis to give the corresponding 2-carboxamides **26** and **27**. Five-membered-ring derivatives were also obtained by Fisher indole synthesis (Scheme 3). Esters **28** and **29** were obtained from ethyl cyclopentanone 2-carboxylate and subjected to microwave-assisted ammonolysis to give the carboxamides **30** and **31**, respectively. The seven-membered-ring derivative **35** was synthesized as shown in Scheme 5. Bromo ketone **33** was cyclized with *p*-chloroaniline to provide ester **34**, followed by ammonolysis to give racemic **35**. Preparative chiral HPLC gave multigram quantities of the enantiomers of **35**. *R*- and *S*-configurations were assigned by X-ray crystallographic analysis.

Results and Discussion

In an effort to discover modulators of human SIRT enzymes, we performed a high-throughput screen of 280 000 compounds against recombinant human SIRT1 using a fluorimetric assay.[29,30] Among the confirmed hits were several indoles, shown in Figure 2. To corroborate data obtained using the fluorimetric assay, we developed a radiochemical assay that monitors enzymatic release of nicotinamide from NAD.[31,32]

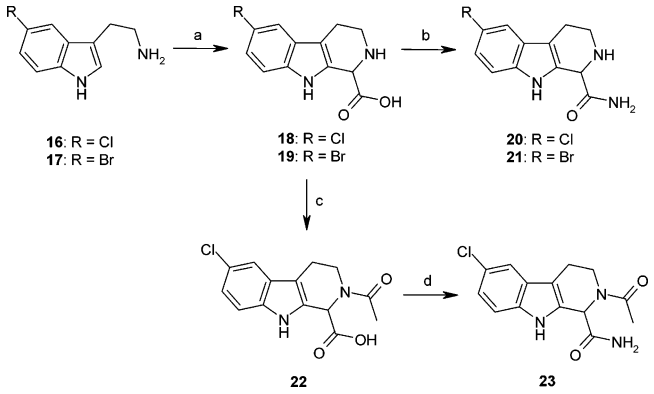
The indoles were found to be active against SIRT1 in both the fluorimetric and radiochemical assays, as shown in Table 1. We observed, however, that the indoles were 2−12-fold more potent in the fluorimetric assay than in the radiochemical assay. These differences in potency may reflect the fact that the fluorimetric assay employed a four-amino-acid peptide labeled with aminomethylcoumarin, whereas the radiochemical assay consisted of an unlabeled 19-amino-acid peptide. Kinetic studies suggest that binding of the inhibitor is favored by the presence of the substrates bound in the enzyme active site, so the nature of the substrates may significantly affect inhibitor potency. Enzyme kinetic analyses using the fluorimetric assay showed compound **1** to be a mixed-type inhibitor against both NAD and acetyl-peptide substrates. The inhibition pattern approached that of an uncompetitive inhibitor, especially against NAD (Figure 3). This suggests that binding of the inhibitor occurs after binding of both substrates,[33] and we speculate that the inhibition occurs after the release of nicotinamide (see Figure 1). To test this hypothesis, we examined the effect of compound **1** on nicotinamide exchange[21−23] using the nicotinamide re-

Scheme 1[a]



[a] Reagents and conditions: (a) Br_2; (b) 150 °C, 3 h; (c) NaOH, 3:1 ethanol−water; (d) *S*-PhCH(Me)NH_2, EDC, HOBt, DCM, rt; (e) H_2SO_4; (f) BH_3−THF, TFA, THF, 0 °C to rt; (g) H_2SO_4; (h) NH_2OH−THP, TEA, EDC; (i) HCl in dioxane/DCM.

Scheme 2[a]



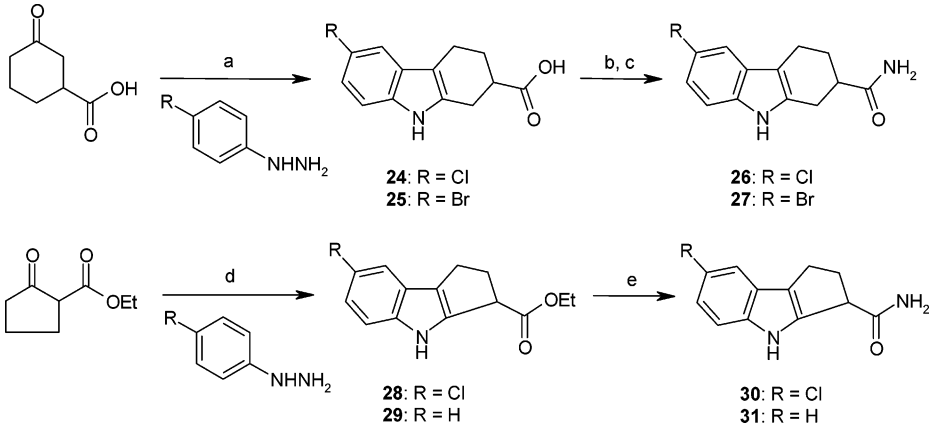
[a] Reagents and conditions: (a) $HCOCO_2H$, KOH; (b) PyAOP, NH_3(aq), DCM/DMF; (c) $(Ac)_2O$, Na_2CO_3, dioxane; (d) NH_3(aq), EDC, HOBt, DCM.

lease assay described previously.[31] SIRT1 was incubated in the presence of a concentration of unlabeled nicotinamide that inhibited the enzyme-catalyzed deacetylation. It has been shown previously that under these conditions release of labeled nicotinamide from NAD results predominantly from nicotinamide exchange, in which the unlabeled nicotinamide is incorporated into NAD.[34,35] Compound **1** inhibited this exchange reaction with an IC_{50} of 1.5 μM, suggesting that the inhibitor may in fact occupy the nicotinamide binding site. As recent kinetic analysis of human SIRT2 showed product release to be the rate-determining step, inhibition of deacetylation by compound **1** may result from prevention of the release of one or both of the products, 2-*O*-acetyl-ADP-ribose and deacetylated peptide.[23]

The most potent compounds inhibited SIRT1 in the fluorimetric assay with IC_{50} values of 60−100 nM, representing a 500-fold improvement over previously reported SIRT inhibitors.[25−28] As shown in Table 1, the indoles displayed a high degree of selectivity for SIRT1 over two other NAD-dependent deacetylases SIRT2 and SIRT3, and these compounds did not inhibit class I and II HDACs nor NAD glycohydrolase (NADase) at a concentration of 100 μM. Additionally, experiments reported elsewhere showed that compound **1** inhibited recombinant SIRT1 expressed and purified from mammalian cells with an IC_{50} of 38 nM, equipotent to its inhibition of SIRT1 expressed in bacteria.[36] Furthermore, compound **1** penetrated cells and inhibited the deacetylation of p53 at a concentration of 1 μM.[36]

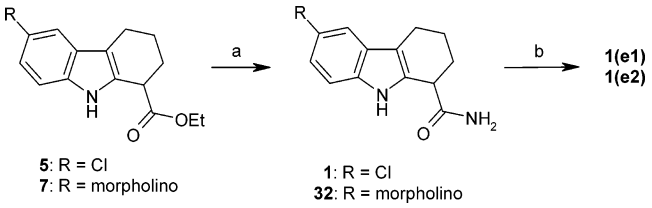
Structure−activity relationships within a series of 6-substituted tetrahydrocarbazoles were explored as shown in Table 2. There is a very strong requirement for the primary carboxamide; *N*-ethyl and *N*,*N*-diethyl carboxamides **36** and **37**, the corresponding carboxylic acid **8**, and the ethyl ester **5** all showed no measurable activity. Further, the hydroxamic acid **15** was 790-fold less potent than the carboxamide **1**. The position of the carboxamide is also important, as evidenced by the 350-fold loss in activity upon moving the carboxamide from the 1-position in compound **1** to the 2-position in compound **26**. Small nonpolar groups were tolerated well at the 6-position in compounds **1**, **2**, and **3**, while a 6-morpholino group in compound **32** was not. Methylation on the carbazole nitrogen in compound **40**, or adjacent to the carboxamide in compound **41**, was not well tolerated. C-ring modifications other than changes to the primary carboxamide are shown in Table 3. The seven-membered-ring compound **35** was equipotent to compound **1**. The five-membered-ring compounds **30** and **31** were 2−4-fold less active than the corresponding tetrahydrocarbazoles **1** and **3**. Compounds containing nitrogen in the C-ring, **20**, **21**, and **23**, retained only marginal activity. Compound **13**, representing a mixture

Scheme 3[a]



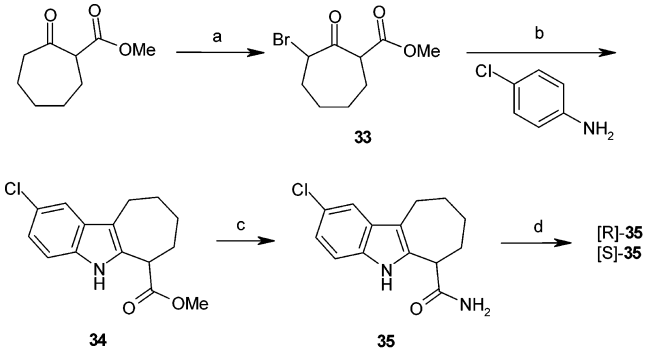
[a] Reagents and conditions: (a) AcOH, reflux; (b) *S*-PhCH(Me)NH_2, EDC, HOBt, DCM; (c) H_2SO_4; (d) TFA, EtOH, reflux; (e) NH_3 in MeOH, microwave.

Scheme 4[a]



[a] Reagents and conditions: (a) NH_3, EtOH, 60 °C, 24 h; (b) Chiralpak AD column.

Scheme 5[a]



[a] Reagents and conditions: (a) Br_2, CCl_4, 4 d; (b) 140 °C; (c) NH_3, MeOH, 90 °C, 48 h; (d) Chiralpak AD column.

of diastereomers formed by reduction to give a fully saturated carbocyclic ring, had no measurable activity. Overall, we observed stringent structural requirements for activity such that little deviation from compounds **1** or **35** is tolerated. The stringency of the structure–activity relationships was further exemplified through the separation and evaluation of the enantiomers of **1** and **35**. In both cases one enantiomer was at least 360-fold more active than the other (Tables 2 and 3).

We used a panel of in vitro assays and in vivo pharmacokinetic studies to assess ADME properties. The results in Table 1 suggest that the indole series exhibit few if any potential liabilities. None of the compounds tested at 1 μM inhibited a panel of cytochrome P450 enzymes, nor did the compounds affect the activity of the hERG channel when tested at 10 μM. Both **1** and **35** were highly stable in rat liver microsomes. Finally, pharmacokinetic analysis of compounds **1** and **35** following oral dosing in mice showed quantitative bioavailability and serum half-lives of 136 and 94 min, respectively. Thus these compounds are well-suited to study SIRT1 inhibition in vivo.

Conclusions

A fluorimetric screen of human SIRT1 led to the discovery of a series of indoles as potent inhibitors that are selective for SIRT1 over other deacetylases and NAD-processing enzymes. Furthermore, these compounds meet many of our criteria for entering lead optimization in that they display a favorable ADME profile, including oral bioavailability and metabolic stability. These indoles represent the most potent SIRT1 inhibitors reported to date, providing a useful set of chemical tools to study the biology of SIRT1 and to explore therapeutic uses for SIRT inhibitors.

Experimental Section

Chemistry. ^{1}H NMR spectra were recorded at 400 MHz on a Bruker AVANCE 400-MHz digital two-channel NMR spectrometer. Compounds were analyzed by HPLC/MS using a Hypersil BDS C18, 2.1 × 50 mm, 5 μm column. Samples were injected in 3 μL volume and eluted at a flow rate of 1 mL/min. A gradient of 0.1% aqueous TFA to 0.1% TFA in 95% acetonitrile was run over 1.8 min, followed by 0.1% TFA in 95% acetonitrile for a further 0.3 min. Peaks were detected by UV absorbance (215 nm) and ELS. Key compounds were further analyzed by a second HPLC method and characterized by high-resolution MS as described in the Supporting Information. Compounds **2**, **3**, **36**, **37**, **40**, and **41** were purchased from Chembridge. Compounds **38** and **39** were purchased from Specs and Asinex, respectively.

Ethyl 3-Bromo-2-oxocyclohexanecarboxylate (4). Ethyl 2-oxocyclohexanecarboxylate (20 g, 0.12 mol) was dissolved in 40 mL of Et_2O and cooled with stirring to 0 °C under N_2. Bromine (6 mL, 0.12 mol) was added dropwise over 15 min, and the reaction mixture was allowed to warm to room temperature over 90 min. The reaction mixture was slowly poured into ice-cold saturated aqueous Na_2CO_3 (150 mL) and extracted with EtOAc (3 × 150 mL). The combined organic layers were dried over Na_2SO_4 and evaporated in vacuo to give 29.2 g (100%) of **4** as a pale yellow oil: ^{1}H NMR (CD_3OD) δ 1.24–1.36 (m, 3H), 1.70–2.53 (m, 7H), 4.15–4.29 (m, 2H), 4.65–4.74 (m, 1H); LCMS t_R = 1.28 min; *m/z* = 251 (100), 253 (100) (MH^+).

Ethyl 6-Chloro-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxylate (5). Keto ester **4** (2.36 g, 9.5 mmol) was added to 4-chloroaniline (3.02 g, 23.75 mmol) and the mixture was heated at 150 °C for 3 h. The reaction mixture was allowed to cool to room temperature and taken up in 100 mL of DCM

Table 1. In Vitro Selectivity and ADME Profile[a]

	IC$_{50}$ (μM)						% inhibition						
	fluorimetric assay				nicotinamide release		at 1 μM						
compd	SIRT1	SIRT2	SIRT3	HDAC	SIRT1	NADase	CYP3A4	CYP2D6	CYP2C9	CYP2C19	CYP1A2	at 10 μM: hERG blockade	half-life (min): rat liver microsomes
1	0.098	19.6	48.7	>100	1.29	>100	−26	−5	−5	6	11	0	>60
2	0.205	11.5	>100	>100	2.5	>100	−16	−3	−9	9	7	0	ND
3	1.47	24.8	>100	>100	3.3	>100	−22	−4	−8	5	4	0	ND
35	0.124	2.77	>100	>100	0.652	>100	−12	3	11	23	23	10	>60

[a] IC$_{50}$ data are reported as the mean of at least three independent determinations; standard error of the mean ≤30%. All other data are the mean of duplicate determinations.

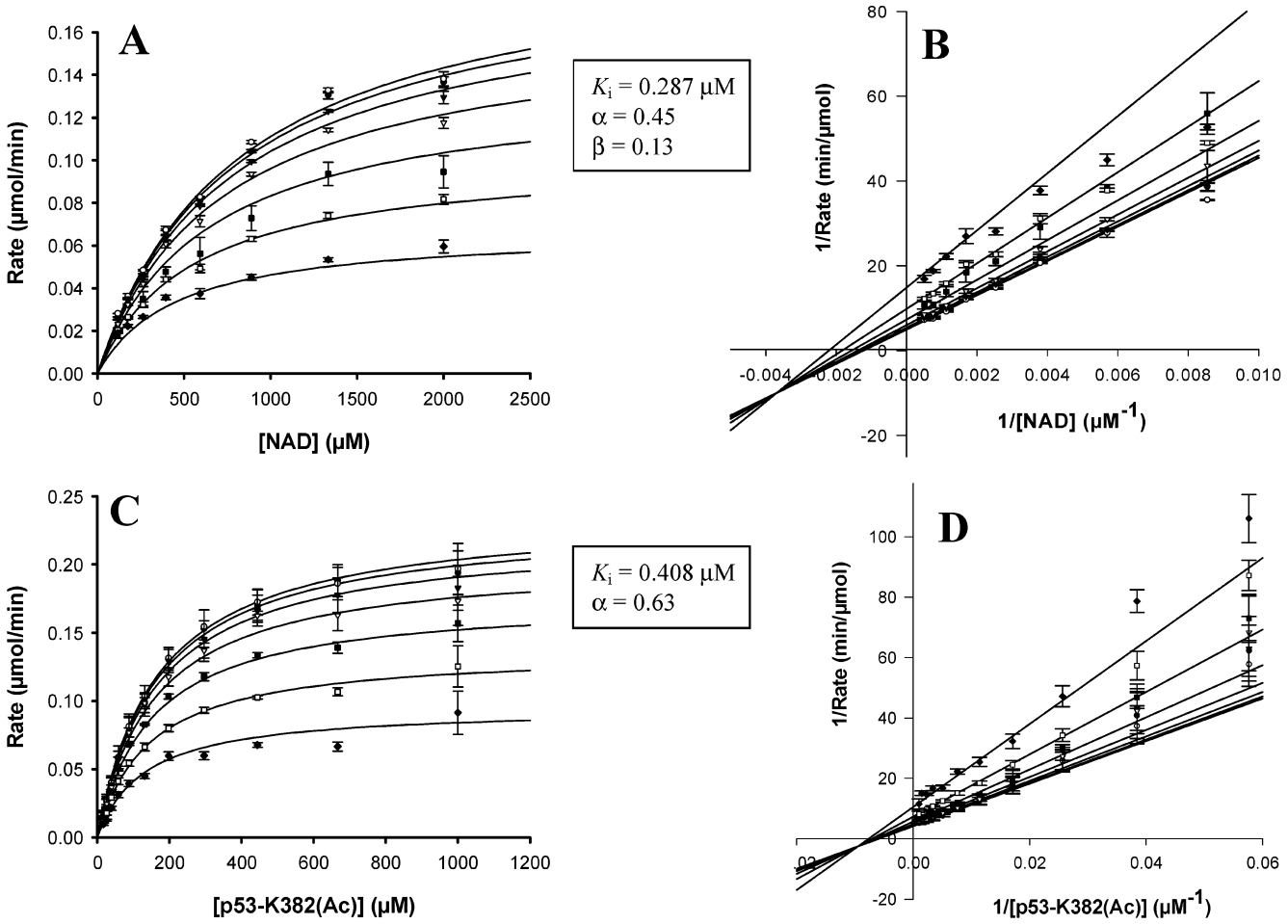


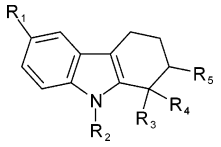
Figure 3. Kinetics of inhibition of SIRT1 by compound **1**. The rate of deacetylation of the p53-Lys^{382} substrate was measured in the SIRT fluorimetric assay in the presence of 0.00625 μM (◆), 0.0125 μM (□), 0.025 μM (■), 0.05 μM (▽), 0.1 μM (▼), 0.2 μM (○), 0.4 μM (●) compound **1**. Inhibition constants, K_i, α, and β,[34] and the pattern of inhibition were determined by direct fit of the data using SigmaPlot (Systat Software, Point Richmond, CA). Panel A shows inhibition by **1** as a function of NAD concentration (100−2000 μM); the concentration of p53-Lys^{382} substrate was fixed at 700 μM. Panel B is a double-reciprocal plot that illustrates the mixed-type inhibition pattern. Panel C shows inhibition by **1** as a function of p53-Lys^{382} concentration (10−1000 μM); the concentration of NAD was fixed at 1000 μM. Panel D is the corresponding double-reciprocal plot.

and washed with 1 N HCl (3 × 100 mL) followed by saturated aqueous $NaHCO_3$ (100 mL). The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude residue was purified by silica gel column chromatography (heptanes/EtOAc 9:1) to give 2.02 g (77%) of **5** as an off white solid: ^{1}H NMR ($CDCl_3$) δ 1.32 (t, J = 7.2 Hz, 3H), 1.75−1.87 (m, 1H), 1.99−2.24 (m, 3H), 2.64−2.71 (m, 2H), 3.79−3.87 (m, 1H), 4.21−4.27 (m, 2H), 7.09 (dd, J = 8.2, 2.1 Hz, 1H), 7.25 (d, J = 8.2 Hz, 1H), 7.44 (bd, J = 2.1 Hz, 1H), 8.40−8.48 (m, 1H); LCMS t_R = 1.72 min; m/z = 278 (100), 280 (33) (MH^+).

Ethyl 6-difluoromethoxy-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxylate (6) was prepared from compound **4** and 4-(difluoromethoxy)aniline according to the method described above for the synthesis of **5**, to give 143 mg (11%) of **6** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.30 (t, J = 7.0 Hz, 3H), 1.78−1.88 (m, 1H), 2.01−2.21 (m, 3H), 2.65−2.71 (m, 2H), 3.79−3.87 (m, 1H), 4.22 (q, J = 7.0 Hz, 2H), 6.49 (t, J = 7.5 Hz, 1H), 6.93 (dd, J = 8.1, 2.0 Hz, 1H), 7.21−7.28 (m, 2H), 8.42 (bs, 1H); LCMS t_R = 1.59 min; m/z = 310 (30) (MH^+), 236 (100).

Ethyl 6-morpholin-4-yl-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxylate (7) was prepared from compound **4** and 4-morpholinoaniline according to the method described above for the synthesis of **5**, to give 130 mg (20%) of **7** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.3 (t, J = 7.1 Hz, 3H), 1.76−1.87 (m, 1H), 2.00−2.10 (m, 1H), 2.13−2.20 (m, 2H), 2.65−2.72 (m, 2H), 3.10−3.16 (m, 4H), 3.79−3.85 (m, 1H), 3.89−3.93 (m, 4H), 4.19−4.26 (m, 2H), 6.91 (dd, J = 8.8, 2.1 Hz, 1H), 7.00 (brd, J

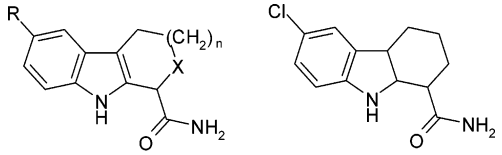
Table 2. Inhibition of SIRT1 by Substituted Tetrahydrocarbazoles



compd	R_1	R_2	R_3	R_4	R_5	IC_{50} (μM)[a]
1	Cl	H	H	$CONH_2$	H	0.098
1(e1)	Cl	H	H	$CONH_2$	H	0.123
1(e2)	Cl	H	H	$CONH_2$	H	>100
2	CH_3	H	H	$CONH_2$	H	0.205
3	H	H	H	$CONH_2$	H	1.47
5	Cl	H	H	CO_2Et	H	>100
8	Cl	H	H	CO_2H	H	>100
12	OH	H	H	$CONH_2$	H	15.0
15	Cl	H	H	CONHOH	H	77.6
26	Cl	H	H	H	$CONH_2$	34.5
27	Br	H	H	H	$CONH_2$	2.44
36	Cl	H	H	CONHEt	H	>100
37	Cl	H	H	$CONEt_2$	H	>100
38	Cl	H	H	$CONHCH_2CONH_2$	H	>100
39	CH_3	H	H	CH_2NH_2	H	>100
40	CH_3	CH_3	H	$CONH_2$	H	>100
41	CH_3	H	CH_3	$CONH_2$	H	13.0

[a] Data are reported as the mean of at least three independent determinations; standard error of the mean ≤30%.

Table 3. Effect of C-Ring Variation on Activity



13

compd	R	*n*	X	IC_{50} (μM)[a]
1	Cl	1	CH_2	0.098
3	H	1	CH_2	1.47
13	–	–	–	>100
20	Cl	1	NH	18.0
21	Br	1	NH	>100
23	Cl	1	$NHCOCH_3$	79.9
30	Cl	0	CH_2	0.409
31	H	0	CH_2	2.67
35	Cl	2	CH_2	0.124
(*S*)-**35**	Cl	2	CH_2	0.063
(*R*)-**35**	Cl	2	CH_2	23.0

[a] Data are reported as the mean of at least three independent determinations; standard error of the mean ≤30%.

= 2.1 Hz, 1H), 7.24 (brd, J = 8.8 Hz, 1H), 8.28 (bs, 1H); LCMS t_R = 1.07 min; m/z = 329 (100) (MH^+).

6-Chloro-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxylic Acid (8). Ester **5** (360 mg, 1.3 mmol) was dissolved in 9 mL of EtOH. NaOH (260 mg, 6.5 mmol) dissolved in H_2O (3 mL) was added and the reaction mixture was stirred at room temperature for 1 h. The EtOH was removed by evaporation and the residue was diluted with H_2O (20 mL). The resultant aqueous solution was washed with DCM (50 mL), acidified to pH 2 with 2 N HCl, and extracted with DCM (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated in vacuo to give 296 mg (91%) of **8** as an off white solid: ^{1}H NMR ($CDCl_3$) δ 1.76−1.92 (m, 1H), 1.96−2.11 (m, 1H), 2.12−2.26 (m, 2H), 2.61−2.75 (m, 2H), 3.51−3.97 (m, 1H), 7.10 (dd, J = 8.6, 1.9 Hz), 7.21 (d, J = 8.6 Hz, 1H), 7.44 (brd, J = 1.9 Hz, 1H), 8.31−8.38 (m, 1H); LCMS t_R = 1.42 min; m/z = 250 (77), 252 (28) (MH^+), 204 (100), 206 (35).

6-Difluoromethoxy-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxylic Acid (9) was prepared from ester **6** according to the method described above for **8**, to give 130 mg (95%) of **9** as a light yellow oil: ^{1}H NMR (CD_3OD) δ 1.68−1.75 (m, 1H), 2.13−2.31 (m, 3H), 2.54−2.68 (m, 2H), 3.71−3.80 (m, 1H), 6.43−6.58 (m, 1H), 6.85 (dd, J = 8.1, 1.9 Hz, 1H), 7.11−7.21 (m, 2H); LCMS t_R = 1.37 min; m/z = 282 (30) (MH^+), 236 (100).

6-Chloro-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxylic Acid (1-Phenylethyl)amide (10). *S*-α-Methylbenzylamine (620 μL, 4.8 mmol) was added to a solution of **8** (1 g, 4 mmol), EDC (921 mg, 4.8 mmol), and HOBt (649 mg, 4.8 mmol) in 20 mL of DCM and stirred overnight at room temperature. The reaction mixture was washed with 1 N HCl (50 mL) and saturated aqueous $NaHCO_3$ (50 mL) and dried over Na_2SO_4. Solvent was evaporated in vacuo, and the residue was purified by silica gel column chromatography (1% MeOH in DCM) to give 900 mg (64%) of **10** as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.35−1.41 (m, 3H), 1.68−2.14 (m, 4H), 2.55−2.67 (m, 2H), 3.59−3.70 (m, 1H), 5.01−5.11 (m, 1H), 5.78−5.89 (m, 1H), 6.98−7.39 (m, 8H), 8.65−8.81 (m, 1H); LCMS t_R = 1.71 min; m/z = 353 (100), 355 (38) (MH^+).

6-Difluoromethoxy-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxylic acid (1-phenylethyl)amide (11) was prepared from carboxylic acid **9** according to the method described above for **10**, to give 25 mg (10%) of **11** as an off-white solid: ^{1}H NMR ($CDCl_3$) δ 1.41−1.52 (m, 3H), 1.88−2.17 (m, 4H), 2.55−2.63 (m, 2H), 3.78−3.90 (m, 1H), 4.97−5.05 (m, 1H), 6.01−6.09 (m, 1H), 6.35 (t, J = 6.8 Hz, 1H), 7.01−7.57 (m, 8H), 8.83−8.88 (m, 1H); LCMS t_R = 1.37 min; m/z = 385 (100) (MH^+).

6-Chloro-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxamide (1). Synthesis from Compound 10. Amide **10** (900 mg, 2.55 mmol) was dissolved in 9 mL of H_2SO_4 and the solution was stirred at room temperature for 2 h. The reaction mixture was cooled to 0 °C and slowly quenched to pH 7 with 5 N NaOH, keeping the temperature below 20 °C. The resultant aqueous mixture was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and evaporated in vacuo to give a pale yellow oil. The residue was purified by silica gel column chromatography (4% MeOH in DCM) to give 450 mg (71%) of **1** as a pale yellow foam: ^{1}H NMR (CD_3OD) δ 1.75−2.30 (m, 4H), 2.61−2.82 (m, 2H), 3.70−3.83 (m, 1H), 6.97−7.09 (m, 1H), 7.19−7.29 (m, 1H), 7.34−7.44 (m, 1H); LCMS t_R = 1.28 min; m/z = 249 (100), 251 (31) (MH^+).

6-Chloro-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxamide (1). Synthesis from Compound 5. NH_3 in EtOH (200 mL of 7 N) was added to a solution of ester **5** (10 g, 40 mmol) in EtOH (100 mL) and the mixture was transferred to a Parr pressure reactor. The reactor was purged to displace air with ammonia and heated to 60 °C for 24 h. Solvent was evaporated in vacuo and the residue was purified on a Biotage 40 L column. After elution with EtOAc, TLC (EtOAc) showed an impurity at slightly higher R_f than the desired product. Further purification was carried out on a second column using a step gradient: Elution with 1:1 heptane/EtOAc removed the impurity, after which 1:4 heptane/EtOAc gave 7.8 g (78%) of **1** as an off-white crystalline solid: ^{1}H NMR (CD_3OD) δ 1.76−2.20 (m, 4H), 2.59−2.75 (m, 2H), 3.69−3.75 (m, 1H), 6.95−7.03 (m, 1H), 7.20−7.24 (m, 1H), 7.34−7.38 (s, 1H); m/z = 249.71 (MH^+).

Separation of Enantiomers. The two enantiomers of **1** were obtained by elution of the racemic mixture from a Chiralpak AD column with 30:70 2-propanol/hexanes: **1(e1)**, white solid, t_R = 10.65; **1(e2)**, white solid, t_R = 20.17 min.

6-Hydroxy-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxamide (12) was prepared as described above for the conversion of **10** to **1**. Thus, acid hydrolysis of amide **11** gave 5 mg (33%) of **12** as a off-white solid: ^{1}H NMR (CD_3OD) δ 1.81−1.84 (m, 1H); 1.95−2.07 (m, 1H); 2.10−2.15 (m, 2H); 2.63−2.69 (m, 2H); 3.71 (t, J = 6.2 Hz, 1H); 6.62 (dd, J = 8.6, 2.3 Hz, 1H); 6.79 (d, J = 2.3 Hz, 1H); 7.11 (d, J = 8.6 Hz, 1H); LCMS t_R = 1.03 min; m/z = 231 (100) (MH^+).

6-Chloro-2,3,4,4a,9,9a-hexahydro-1*H*-carbazole-1-carboxamide (13).[37] At 0 °C, borane−THF complex (4.4 mL, 1

M in THF) was added dropwise to a solution of 2.2 mL of TFA in 2.2 mL of dry THF and the mixture stirred for 5 min. A solution of **10** (100 mg, 0.29 mmol) in 0.5 mL of THF was added, and the mixture was stirred for 2.5 h at room temperature. Water (15 mL) was added slowly, and the resulting mixture was basified with 1 N NaOH to pH 10 and extracted with DCM (3 × 50 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by silica gel column chromatography (10% MeOH in DCM) to give 74 mg (74%) of **6-chloro-2,3,4,4a,9,9a-hexahydro-1*H*-carbazole-1-carboxylic acid (1-phenylethyl)amide:** LCMS t_R = 1.39 min; *m/z* = 355 (100), 357 (35) (MH^+).

The above amide intermediate was deprotected as for **1** to give 26 mg (50%) of **13** as a white solid: ^{1}H NMR (CD_3OD) δ 1.04−1.23 (m, 2H), 1.28−1.48 (m, 1H), 1.71−1.84 (m, 4H), 2.61−2.69 (m, 1H), 2.91−3.01 (m, 1H), 3.97−4.01 (m, 1H), 6.65 (d, *J* = 8.2 Hz, 1H), 6.95 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.04 (d, *J* = 2.1 Hz, 1H); LCMS t_R = 0.97 min; *m/z* = 251 (100), 253 (32) (MH^+), 234 (31), 236 (9).

6-Chloro-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxylic Acid (Tetrahydropyran-2-yloxy)amide (14). To carboxylic acid **8** (110 mg, 0.43 mmol) in 2 mL of DMF were added triethylamine (90 μL, 0.65 mmol), *O*-THP-hydroxylamine (130 mg, 1.1 mmol), and EDC (100 mg, 0.52 mmol). The resulting mixture was stirred overnight. Solvent was evaporated in vacuo and the residual oil was dissolved in EtOAc (10 mL), washed with 1 N HCl (3 × 10 mL), and dried over $MgSO_4$. The organic layer was evaporated in vacuo and the residue was purified by silica gel column chromatography (EtOAc/heptane 1:1) to give 15 mg (10%) of **14** as a light yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.16−1.75 (m, 8H), 1.93−2.01 (m, 1H), 2.03−2.10 (m, 1H), 2.55−2.64 (m, 2H), 3.42−3.55 (m, 1H), 3.59−3.67 (m, 1H), 3.79−3.88 (m, 1H), 4.86−4.93 (m, 1H), 6.98−7.03 (m, 1H), 7.08−7.11 (m, 1H), 7.33−7.40 (m, 1H), 8.51−8.61 (m, 1H), 8.76−8.85 (m, 1H); LCMS t_R = 1.45 min; *m/z* = 371 (15), 373 (5) ($M + Na^+$), 265 (22), 267 (8), 204 (100), 206 (34).

6-Chloro-2,3,4,9-tetrahydro-1*H*-carbazole-1-hydroxyamic Acid (15). *O*-THP-hydroxamic acid **14** (15 mg, 0.04 mmol) was dissolved in 1 mL of DCM, 4 N HCl in dioxane (20 μL, 0.08 mmol) was added, and the reaction mixture was stirred at room temperature for 2 h. Solvent was evaporated in vacuo and the residue was purified by silica gel column chromatography (EtOAc/heptane 1:1) to give 10 mg (88%) of **15** as a colorless oil: ^{1}H NMR (CD_3OD) δ 1.59−1.71 (m, 1H), 1.86−2.01 (m, 3H), 2.58−2.65 (m, 2H), 3.55−3.60 (m, 1H), 6.90 (dd, *J* = 8.6, 2.0 Hz, 1H), 7.11 (d, *J* = 8.6 Hz, 1H), 7.41 (d, *J* = 2.0 Hz, 1H); LCMS t_R = 1.64 min; *m/z* = 265 (100), 267 (32) (MH^+).

6-Chloro-2,3,4,9-tetrahydro-1*H*-β-carboline-1-carboxylic Acid (18).[38] A solution of glyoxylic acid (110 mg, 1.2 mmol) in water (1 mL) followed by 1 N KOH (1 mL) was added dropwise to a suspension of **16** (210 mg, 1.07 mmol) in 5:1 water/dioxane (15 mL). The reaction mixture was stirred at room temperature overnight. The precipitate was filtered, washed with water, and dried in a vacuum oven to give 63 mg (23%) of **18** as an off-white powder: ^{1}H NMR (DMSO-d_6) δ 2.74−2.86 (m,1H), 2.87−2.97 (m, 1H), 3.23−3.43 (m, 2H), 4.70 (s, 1H), 7.03 (dd, *J* = 2.0, 8.6 Hz, 1H), 7.43 (brd, *J* = 2.0 Hz, 1H), 7.46 (d, *J* = 8.6 Hz, 1H), 8.81−9.20 (m, 1H), 10.97 (bs, 1H).

6-Bromo-2,3,4,9-tetrahydro-1*H*-β-carboline-1-carboxylic acid (19) was prepared according to the method described above for compound **18**. Thus indole **17** gave 210 mg (67%) of **19** as an off-white solid: ^{1}H NMR (DMSO-d_6) δ 2.77−2.89 (m, 2H), 3.28−3.49 (m, 2H), 4.72 (s, 1H), 7.15 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.43 (d, *J* = 8.6 Hz, 1H), 7.58 (d, *J* = 1.9 Hz, 1H), 10.98 (bs, 1H).

6-Chloro-2,3,4,9-tetrahydro-1*H*-β-carboline-1-carboxamide (20). Carboxylic acid **18** (20 mg, 0.08 mmol) was dissolved in 4:1 DCM/DMF (2 mL), and PyAOP (50 mg, 0.1 mmol) was added. After stirring of the reaction mixture for 30 min, 30% aqueous NH_4OH (20 μL) was added, and the resulting mixture was stirred at room temperature overnight. Solvent was evaporated in vacuo and the residue was dissolved in EtOAc (5 mL), washed with saturated $NaHCO_3$ (2 × 5 mL), and dried over Na_2SO_4. The organic layer was evaporated in vacuo, and the residue was purified by silica gel column chromatography (DCM/MeOH/NH_4OH 97.5/2.5/0.1 to 90/10/0.1) to give 10 mg (50%) of **20** as an off-white solid: ^{1}H NMR (CD_3OD) δ 3.03−3.28 (m, 2H), 3.60−3.69 (m, 1H), 3.79−3.88 (m, 1H), 5.41 (s, 1H), 7.21 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.44 (d, *J* = 8.8 Hz, 1H), 7.55 (brd, *J* = 2.1 Hz, 1H); LCMS t_R = 1.43 min; *m/z* = 250 (100), 252 (39) (MH^+).

6-Bromo-2,3,4,9-tetrahydro-1*H*-β-carboline-1-carboxamide (21) was prepared according to the method described above for compound **20**. Thus carboxylic acid **19** gave 23 mg (45%) of **21** as an off-white solid: ^{1}H NMR (CD_3OD) δ 2.91−3.10 (m, 2H), 3.48−3.59 (m, 1H), 3.68−3.77 (m, 1H), 5.29 (s, 1H), 7.23 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.29 (d, *J* = 8.5 Hz, 1H), 7.61 (brd, *J* = 1.7 Hz, 1H); LCMS t_R = 1.43 min; *m/z* = 294 (98), 296 (100) (MH^+).

2-Acetyl-6-chloro-2,3,4,9-tetrahydro-1*H*-β-carboline-1-carboxylic Acid (22). Carboxylic acid **18** (250 mg, 1 mmol) was dissolved in dioxane (5 mL). A solution of Na_2CO_3 (210 mg, 2 mmol) in water (5 mL) and acetic anhydride (186 μL, 2 mmol) were added, and the reaction mixture was stirred overnight at room temperature. Solvent was evaporated in vacuo, and the residue was dissolved in EtOAc (30 mL), washed with 0.5 N HCl (15 mL), and dried over Na_2SO_4. The EtOAc solution was evaporated in vacuo to give 160 mg (55%) of **22** as a yellow oil, which was used without further purification.

2-Acetyl-6-chloro-2,3,4,9-tetrahydro-1*H*-β-carboline-1-carboxamide (23). Carboxylic acid **22** (50 mg, 0.17 mmol), EDC (50 mg, 0.26 mmol), and HOBt (35 mg, 0.26 mmol) were dissolved in DCM (2 mL), and the resulting mixture was stirred for 1 h. NH_4OH (0.24 mL, 28% in water) was added, and the reaction mixture was stirred overnight. Solvent was evaporated in vacuo, and the residue was dissolved in EtOAc (3 mL), washed with water (2 × 2 mL), and dried over Na_2SO_4. The EtOAc solution was evaporated in vacuo and the residue was purified by HPLC on a Hyperprep HS C18 column (0.1% TFA in 20% aqueous acetonitrile to 0.1% TFA in acetonitrile over 7.5 min) to give 8 mg (16%) of **23** as a colorless oil: ^{1}H NMR (CD_3OD) δ 2.30 (s, 3H), 2.78−2.88 (m, 2H), 3.58−3.69 (m, 1H), 4.17−4.28 (m, 1H), 6.01 (s, 1H), 7.06 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.32 (d, *J* = 8.5 Hz, 1H), 7.42 (brd, *J* = 2.0 Hz, 1H); LCMS t_R = 1.15 min; *m/z* = 292 (100), 294 (35) (MH^+).

6-Chloro-2,3,4,9-tetrahydro-1*H*-carbazole-2-carboxylic Acid (24).[39] 4-Chlorophenylhydrazine hydrochloride (240 mg, 1.34 mmol) and cyclohexanone-3-carboxylic acid (200 mg, 1.41 mmol) were added to AcOH (4 mL). The resulting mixture was stirred at room temperature for 1 h and then refluxed for 5 h. Ice water (10 mL) was added, and the mixture was cooled at 0 °C. The precipitate was filtered and recrystallized from EtOH to yield 48 mg (14%) of **24** as white crystals: ^{1}H NMR (DMSO-d_6) δ 1.76−1.87 (m, 1H), 2.09−2.19 (m, 1H), 2.54−2.66 (m, 1H), 2.67−2.83 (m, 1H), 2.86−2.93 (m, 1H), 2.83−2.89 (m, 2H), 6.97 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.26 (d, *J* = 8.2 Hz, 1H), 7.36 (brd, *J* = 2.0 Hz, 1H), 10.93 (bs, 1H), 12.33 (m, 1H); LCMS t_R = 1.39 min; *m/z* = 250 (100), 252 (31) (MH^+).

6-Bromo-2,3,4,9-tetrahydro-1*H*-carbazole-2-carboxylic acid (25) was prepared according to the method described above for compound **24**. Thus, 4-bromophenylhydrazine hydrochloride gave 350 mg (89%) of **25** as a white solid: ^{1}H NMR (DMSO-d_6) δ 1.73−1.84 (m, 1H), 2.10−2.16 (m, 1H), 2.52−2.58 (m, 1H), 2.61−2.68 (m, 1H), 2.72−2.81 (m, 1H), 2.83−2.89 (m, 2H), 7.03 (dd, *J* = 8.1, 1.9 Hz, 1H), 7.18 (d, *J* = 8.1 Hz, 1H), 7.44 (brd, *J* = 1.9 Hz, 1H), 10.91 (bs, 1H), 12.27 (m, 1H); LCMS t_R = 1.49 min; *m/z* = 294 (100), 296 (99) (MH^+).

6-Chloro-2,3,4,9-tetrahydro-1*H*-carbazole-2-carboxamide (26). EDC (44 mg, 0.23 mmol) was added to a suspension of **24** (48 mg, 0.19 mmol) and HOBt (31 mg, 0.23 mmol) in DCM (5 mL) at 0 °C and the mixture was stirred for 5 min. *S*-α-Methylbenzylamine (30 μL, 0.23 mmol) was added and the reaction mixture was stirred overnight at room temperature. Appearance of product was monitored by TLC (R_f = 0.30,

EtOAc/heptane 1:1). The reaction mixture was washed with saturated aqueous citric acid (5 mL) and dried over Na_2SO_4. The organic phase was evaporated in vacuo and the residue was purified by silica gel column chromatography (EtOAc/heptane 3:7) to give 60 mg (88%) of the desired **6-Chloro-2,3,4,9-tetrahydro-1*H*-carbazole-2-carboxylic acid (1-phenylethyl)amide** as a colorless oil.

H_2SO_4 (1 mL) was added to the above α-methylbenzylamide (60 mg, 0.17 mmol) and the reaction mixture was stirred at room temperature for 2 h. Ice water (5 mL) was carefully added, and the aqueous mixture was extracted with DCM (2 × 10 mL). The combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by silica gel column chromatography (MeOH/DCM 5:95) to give 18 mg (42%) of **26** as a white powder. TLC $R_f = 0.23$ (MeOH/DCM 5:95); ^{1}H NMR (DMSO-d_6) δ 1.65−1.79 (m, 1H), 20.2−2.11 (m, 1H), 2.54−2.66 (m, 2H), 2.69−2.90 (m, 3H), 6.90 (bs, 1H), 6.97 (dd, $J = 8.6$, 2.1 Hz, 1H), 7.25 (d, $J = 8.6$ Hz, 1H), 7.36 (brd, $J = 2.2$ Hz, 1H), 7.43 (bs, 1H), 10.92 (bs, 1H); LCMS $t_R = 1.38$ min; $m/z = 249$ (100), 251 (33) (MH^+).

6-Bromo-2,3,4,9-tetrahydro-1*H*-carbazole-2-carboxamide (27) was prepared from carboxylic acid **25** according to the method described above for compound **26**. Condensation of acid **25** with *S*-α-methylbenzylamine gave the desired amide (TLC $R_f = 0.48$, EtOAc/heptane 7:3). Removal of the α-methylbenzyl group gave 18 mg (36%) of **27** as a white powder: TLC $R_f = 0.25$ (MeOH/DCM 5:95); ^{1}H NMR (DMSO-d_6) δ 1.61−1.75 (m, 1H), 1.98−2.07 (m,1H), 2.49−2.62 (m, 2H), 2.65−2.85 (m, 3H), 6.86 (bs, 1H), 7.05 (dd, $J = 8.6$, 1.9 Hz, 1H), 7.17 (d, $J = 8.6$ Hz, 1H), 7.39 (bs, 1H), 7.45 (brd, $J = 1.9$ Hz, 1H), 10.89 (bs, 1H); LCMS $t_R = 1.50$ min, $m/z = 293$ (100), 295 (99) (MH^+).

Ethyl 7-Chloro-1,2,3,4-tetrahydrocyclopenta[*b*]indole-3-carboxylate (28). 4-Chlorophenylhydrazine hydrochloride (500 mg, 2.8 mmol) was added to a solution of ethyl cyclopentanone-2-carboxylate (440 mg, 2.8 mmol) in EtOH (10 mL). TFA (2 mL) was added, and the reaction mixture was refluxed for 2 h. After evaporation of solvent in vacuo, the residue was dissolved in DCM (10 mL), washed with 1 N HCl, and dried over $MgSO_4$. The DCM solution was evaporated in vacuo, and the residue was purified by silica gel column chromatography (EtOAc/heptane 1:9) to give 90 mg (12%) of **28** as a light yellow oil: TLC $R_f = 0.23$ (EtOAc/heptane 1:9); ^{1}H NMR ($CDCl_3$) δ 1.29 (t, $J = 7.1$ Hz, 3H), 2.69−2.92 (m, 4H), 4.02−4.11 (m, 1H), 4.15−4.23(m, 2H), 7.02 (dd, $J = 8.3$, 2.0 Hz, 1H), 7.21 (d, $J = 8.3$ Hz, 1H), 7.41 (brd, $J = 2.0$ Hz, 1H), 8.17 (bs, 1H); LCMS $t_R = 1.76$ min; $m/z = 264$ (100), 266 (33) (MH^+).

Ethyl 1,2,3,4-tetrahydrocyclopenta[*b*]indole-3-carboxylate (29) was prepared according to the method described above for compound **28**. Thus, phenylhydrazine hydrochloride gave 120 mg (12%) of **29** as a white solid; ^{1}H NMR (CD_3OD) δ 1.20 (t, $J = 7.3$ Hz, 3H), 2.61−2.72 (m, 3H), 2.75−2.84 (m, 1H), 3.91−4.00 (m, 1H), 4.12 (q, $J = 7.3$ Hz, 2H), 6.81−6.99 (m, 2H), 7.17−7.26 (m, 2H); LCMS $t_R = 1.49$ min; $m/z = 230$ (100) (MH^+).

7-Chloro-1,2,3,4-tetrahydrocyclopenta[*b*]indole-3-carboxamide (30). Ethyl ester **28** (50 mg, 0.19 mmol) was dissolved in NH_3 in MeOH (3 mL, 7 N). The reaction mixture was heated in a focused microwave (110 °C, 150 W, 250 psi) for 3 h. Solvent was evaporated in vacuo and the residual product was recrystallized from EtOH to give 10 mg (23%) of **30** as white crystals: ^{1}H NMR (DMSO-d_6) δ 2.52−2.71 (m, 3H), 2.75−2.83 (m, 1H), 3.88−3.95 (m, 1H), 7.01 (dd, $J = 8.4$, 1.9 Hz, 1H), 7.09 (bs, 1H), 7.29 (d, $J = 8.4$ Hz, 1H), 7.36 (brd, $J = 1.9$ Hz, 1H), 7.46 (bs, 1H), 11.02 (bs, 1H); LCMS $t_R = 1.28$ min; $m/z = 235$ (100), 237 (31) (MH^+).

1,2,3,4-Tetrahydrocyclopenta[*b*]indole-3-carboxamide (31) was prepared from ester **29** according to the method described above for compound **30**. Recrystallization from ethanol gave 24 mg (55%) of **31** as white crystals: ^{1}H NMR (DMSO-d_6) δ 2.56−2.74 (m, 3H), 2.77−2.86 (m, 1H), 3.88−3.93 (m, 1H), 6.90−7.08 (m, 3H), 7.27−735 (m, 2H), 7.38−7.43 (m, 1H), 10.77 (bs, 1H); LCMS $t_R = 1.62$ min; $m/z = 201$ (100) (MH^+).

6-Morpholin-4-yl-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxamide (32). Ester **7** (10 mg, 0.03 mmol) was dissolved in 1 mL of 7 N ammonia in methanol. The reaction mixture was heated at 50 °C overnight in a sealed tube. Solvent was evaporated in vacuo and the oily residue was purified by silica gel column chromatography (EtOAc/heptane 8:2) to give 8 mg (84%) of **32** as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.84−2.03 (m, 2H), 2.12−2.21 (m, 2H), 2.70−2.77 (m, 2H), 3.10−3.17 (m, 4H), 3.70−3.76 (m, 1H), 3.89−3.95 (m, 4H), 5.59−5.71 (m, 2H), 6.94 (bd, $J = 8.1$ Hz, 1H), 7.01 (bs, 1H), 7.25 (d, $J = 8.1$ Hz, 1H), 8.29 (bs, 1H); LCMS $t_R = 0.99$ min; $m/z = 300$ (100) (MH^+).

Methyl 3-Bromo-2-oxo-1-cycloheptanecarboxylate (33). Methyl 2-oxo-1-cycloheptanecarboxylate (50 g, 294 mmol) was dissolved in CCl_4 (200 mL) and the solution was chilled to 0 °C. Bromine (46.8 g, 294 mmol) was added dropwise with cooling over 30 min. The reaction mixture was allowed to warm to room temperature and stirred for 4 d. The resulting solution was washed with water (2 × 1 L) and the organic layer was dried over Na_2SO_4 and evaporated in vacuo to give 72.4 g (99%) of **33** as a pale yellow oil. This material was used without further purification.

Methyl 2-Chloro-5,6,7,8,9,10-hexahydro-cyclohepta[*b*]-indole-6-carboxylate (34). Keto ester **33** (16.8 g, 67.4 mmol) and 4-chloroaniline (18.4 g, 144 mmol) were mixed and heated. As the temperature passed 140 °C vigorous evolution of gas occurred, and the reaction mixture was cooled with water immediately. The cooled mixture was dissolved in DCM (200 mL), and the resulting solution was washed with water (2 × 50 mL), aqueous HCl (3 × 50 mL), water (2 × 50 mL), and brine (50 mL). The organic layer was dried over Na_2SO_4, and solvent was evaporated in vacuo. The residue was purified on a Biotage column (heptane/EtOAc 9:1) to give 10 g (53%) of **34** as an off-white solid. This material was used without further purification.

2-Chloro-5,6,7,8,9,10-hexahydro-cyclohepta[*b*]indole-6-carboxylic Acid Amide (35). Ester **34** (10 g, 36 mmol) was dissolved in 7 N ammonia in methanol (350 mL) and the solution was transferred to a Parr pressure reactor. The reaction vessel was purged with N_2 and heated to 90 °C for 48 h. The reaction mixture was cooled to room temperature and solvent was evaporated in vacuo. The residue was purified on a Biotage column (heptane/EtOAc 1:1 followed by EtOAc) to give an off-white foam. Trituration with DCM gave 2 g (21%) of **35** as an off-white solid: ^{1}H NMR (CD_3OD) δ 1.55−1.58 (m, 1H), 1.81−1.99 (m, 5H), 2.38−2.40 (m, 1H), 2.64−2.73 (m, 1H), 2.88−2.91 (m, 1H), 3.83−3.86 (m, 1H), 6.98−7.01 (m, 1H), 7.21−7.24 (m, 1H), 7.40−7.41 (m, 1H); $m/z = 263$ (M^+).

Separation of Enantiomers. The two enantiomers of **35** were obtained by elution of the racemic mixture from a Chiralpak AD-H column with 32% 2-propanol/CO_2 at 200 bar: (*S*)-**35**, white crystalline solid, $t_R = 5.0$ min; (*R*)-**35**, white crystalline solid, $t_R = 5.9$ min. *R*- and *S*-configurations were assigned on the basis of X-ray crystallographic analyses as described in Supporting Information.

SIRT Fluorimetric Assay. Human SIRT1 (N-terminal His6-tag) and SIRT2 (N-terminal GST tag) were expressed in *Escherichia coli* and purified by affinity chromatography.[29] SIRT3 is synthesized in mammalian cells as an inactive precursor and activated by a mitochondrial matrix processing peptidase, which removes the first 100 amino acids.[3] Therefore, N-terminal GST-tagged SIRT3 lacking the first 100 amino acids was expressed in bacteria.

The biochemical assay for recombinant human SIRTs was based on the SIRT1 Fluorimetric Drug Discovery Kit (AK-555, Biomol, Plymouth Meeting, PA). In this assay, modified acetyl lysine conjugated to aminomethylcoumarin (AMC) is deacetylated by SIRT enzymes in the presence of NAD, followed by the addition of a proteolytic developer that releases the fluorescent AMC.[30] K_M values for NAD and acetylated peptide substrates with SIRT1, SIRT2, and SIRT3 were determined as previously reported.[29] Deacetylase reactions were carried out with all substrates fixed at 70% of their K_M values. Enzyme was added at a concentration of 0.5 to 1.0 units/assay well (1

unit of enzyme releases 1 pmol of product per min). Assays were incubated for 45 min at 37 °C. Resulting fluorescence was measured after further incubation for 15 min at 37 °C with Fluor de Lys Developer II (KI-176, Biomol) on a Bio-Tek Synergy HT fluorimeter with excitation set at 360 nm and emission measured at 460 nm. Assays of SIRT1, SIRT2, and SIRT3 were performed in 384-well plates using the p53-K382-(Ac) substrate representing residues 379−382 of p53 acetylated on lysine 382 (KI-177, Biomol). IC_{50} data were analyzed using XLFit (IDBS, Guildford, UK) or GraphPad Prism software.

Class I and II HDAC Fluorimetric Assay. Class I and II HDAC deacetylase activities were measured in the above fluorimetric assay using a class I and II HDAC-containing HeLa cell extract (Biomol) and H4-K16(Ac) substrate representing residues 12−16 of histone H4 acetylated on lysine 16 (KI-174, Biomol).

Nicotinamide Release Assay. The activity of SIRT1 was measured in a nonfluorimetric assay using a p53 peptide substrate representing residues 368−386 acetylated on lysine 382 (Quality Controlled Biochemicals, Hopkinton, MA). This assay measures the release of [^{14}C]nicotinamide from [carbonyl-^{14}C]-NAD (CFA372, Amersham Biosciences, Piscataway, NJ), as previously described.[31,32]

Nicotinamide exchange was measured using the assay as described above in the presence of unlabeled nicotinamide added to a concentration of 52 μM. The added nicotinamide promotes release of [^{14}C]nicotinamide from the labeled NAD through enzyme-catalyzed exchange. After release of [^{14}C]-nicotinamide from NAD, unlabeled nicotinamide binds to the enzyme and is converted to unlabeled NAD.[34,35]

NAD glycohydrolase (NADase) enzymatic activity was measured in the nicotinamide release assay as described above. Crude NADase fraction from pig brain (N9879, Sigma) was purified by anion exchange chromatography. Each assay well contained 0.5 μg of purified enzyme and NAD at a concentration of 18.55 μM (70% of K_M).

Microsomal Stability. In vitro metabolic stability was assessed using rat hepatic microsomes (BD GenTest, Bedford, MA). Compounds at a concentration of 10 μM were incubated at 37 °C with rat hepatic microsomes (1 mg of protein/mL) and quantified by HPLC/MS after 0, 5, 15, 30, and 60 min. Control incubations contained no microsomes.

Cytochrome P450 Inhibition Assays. Cytochrome P450 assays were performed in a 384-well microplate format using recombinant human isozymes 3A4, 2D6, 1A2, 2C9, and 2C19 incubated with fluorogenic substrates as previously reported.[40]

hERG Assay. Chinese hamster ovary (CHO) cells were stably transfected with the hERG potassium channel. Blockade of the hERG channel gives rise to a change in membrane potential that is measured using a potentiometric dye. Dye-loaded cells were incubated with 10 μM compound and 2 mM potassium chloride. Changes in fluorescence were measured in 384-well microplate format using a Tecan Safire fluorescence reader. The effect of compound on control CHO cells lacking the hERG channel was measured and used to correct for nonspecific quenching and toxicity.

In Vivo Pharmacokinetic Analysis. C57bl/6J mice were dosed intravenously (iv) or by oral gavage with 10 mg/kg of compound **1** or **35** in phosphate-buffered saline containing 4% DMSO and 10% cyclodextrin. Plasma was collected at 5, 15, 30, 60, and 90 min and 2, 4, 6, 8, and 24 h after dosing. Samples were analyzed by LCMS at Absorption Systems (Exton, PA). Plasma samples were prepared by solid-phase extraction in a 96-well plate format. A 50-μL aliquot of plasma was combined with 300 μL of 1% phosphoric acid spiked with an internal standard (warfarin at 50 ng/mL). Plasma samples were transferred to a Waters Oasis HLB 30 mg extraction plate, washed with 5% methanol/water, and eluted with acetonitrile. The elute was evaporated to dryness under N_2 at 37 °C and redissolved in 20% aqueous acetonitrile.

Samples (25 μL) were injected onto a Keystone Hypersil BDS C18, 30 × 2.1 mm, 3 μm column and eluted at 0.3 mL/min. A gradient of 2.5 mM NH_4OH−formic acid (pH 3.5) to 2.5 mM NH_4OH−formic acid in 90% acetonitrile was run over 3 min. Mass spectra were acquired using a PE Sciex API4000 with electrospray interface. Quantification was performed against calibration curves generated by spiking compound **1** or **35** into blank heparinized male C57bl/6J mouse plasma (0.3, 1, 3, 10, 30, 100, 300, 1000 ng/mL final concentration). Percent oral bioavailability was calculated from the ratio of the area under the curve up to the last quantifiable time point after oral and iv dosing, respectively. Terminal elimination half-life was calculated from the data obtained after iv dosing.

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Supporting Information Available: HPLC and HRMS for compounds **1**, **5**, **8**, **12**, **13**, **20**, **21**, **23**, **26**, **27**, **30**, **31**, and **35**. X-ray crystallographic data for *R*- and *S*-enantiomers of compound **35**. This material is available free of charge via the Internet at http://pubs.acs.org.

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