Synthesis and Evaluation of Substituted Chroman-4-one and Chromone Derivatives as Sirtuin 2-Selective Inhibitors

Maria Fridén-Saxin,† Tina Seifert,† Marie Rydén Landergren,§ Tiina Suuronen,‖ Maija Lahtela-Kakkonen,⊥ Elina M. Jarho,*⊥ and Kristina Luthman*†

†Department of Chemistry and Molecular Biology, Medicinal Chemistry, University of Gothenburg, SE-412 96 Göteborg, Sweden
§Department of Medicinal Chemistry, R&D iMed, AstraZeneca R&D, SE-431 83 Mölndal, Sweden
‖School of Pharmacy and Department of Neurology, Institute of Clinical Medicine, University of Eastern Finland, P.O. Box 1627, 70211 Kuopio, Finland

Supporting Information

ABSTRACT: A series of substituted chromone/chroman-4-one derivatives has been synthesized and evaluated as novel inhibitors of SIRT2, an enzyme involved in aging-related diseases, e.g., neurodegenerative disorders. The analogues were efficiently synthesized in a one-step procedure including a base-mediated aldol condensation using microwave irradiation. The most potent compounds, with inhibitory concentrations in the low micromolar range, were substituted in the 2-, 6-, and 8-positions. Larger, electron-withdrawing substituents in the 6- and 8-positions were favorable. The most potent inhibitor of SIRT2 was 6,8-dibromo-2-pentylchroman-4-one with an IC\textsubscript{50} of 1.5 \( \mu \)M. The synthesized compounds show high selectivity toward SIRT2 over SIRT1 and SIRT3 and represent an important starting point for the development of novel SIRT2 inhibitors.

INTRODUCTION

Sirtuins (SIRTs) belong to class III histone deacetylases (HDACs) and function as deacetylating enzymes on lysine residues of various histones and nonhistone substrates with the special requirement for nicotinamide adenine dinucleotide (NAD\textsuperscript{+}) as cosubstrate for their activity.1,2 The SIRTs are a conserved class of enzymes from bacteria to humans. The mammalian sirtuin family consists of seven enzymes (SIRT1–SIRT7) localized in different parts of the cell.3 The sirtuins have become highly interesting targets in drug design as they are involved in important cellular processes such as aging and hence in neurodegenerative disorders such as Parkinson’s, Alzheimer’s, or Huntington’s disease.7–11 SIRTs are also considered to be involved in other age-related diseases such as diabetes mellitus and cancer.13,14 SIRT2 in particular is involved in cell cycle regulation; inhibition of SIRT2 leads to hyperacetylation of \( \alpha \)-tubulin and as a consequence to an inhibition of tumor growth.15 Other studies have linked SIRT2 activity to Parkinson’s disease,16 and reports have shown that SIRT2 inhibition appears to lead to a decreased neuronal cell death.17 Research particularly focused on SIRT2 has resulted in the discovery of a number of potent SIRT2-selective inhibitors such as bis(indolyl)maleimide-based kinase inhibitors,20 coumarin-based compounds,21 the benzothiazole AC-93253,22 cambinol derivatives,23 and the sulfobenzoic acid AK-724 (Chart 1).
Chromones and chroman-4-ones constitute a naturally occurring class of substances which are classified as privileged structures, as compounds based on these scaffolds display a wide range of biological activities defined by the substitution pattern of the scaffold. We have put considerable effort into the development of synthetic strategies for this class of compounds resulting, for example, in an efficient synthetic route to 2-alkyl-substituted chroman-4-ones. The incorporation of various functional groups to furnish highly substituted structures have successfully been conducted through different Pd-mediated cross-coupling reactions, through Mannich reactions, and via a SmI2-mediated Reformatsky type reaction. Recently, we have also developed chromone/chroman-4-one-based β-turn peptidomimetics. In the present study we report substituted chromone and chroman-4-one derivatives as potent and highly selective SIRT2 inhibitors.

RESULTS AND DISCUSSION

Characterization of the Lead Compound. In an initial study, a set of compounds based on the chromone and chroman-4-one scaffolds were tested against human SIRT2 to see if these privileged structures could serve as scaffolds for sirtuin inhibitors or activators (data not shown). Interestingly, 8-bromo-6-chloro-2-pentylchroman-4-one 1a gave excellent inhibition (88%) in a preliminary test at 200 μM concentration in a fluorescence-based assay. A more detailed determination of the inhibitory activity gave an IC50 value of 4.5 μM. Compound 1a was also tested against SIRT1 and SIRT3 at 200 μM concentration resulting in less than 10% inhibition of these sirtuin subtypes (see Supporting Information). As 1a turned out to be a novel potent and highly selective SIRT2 inhibitor, it was chosen for further structure–activity studies.

Compound 1a structurally also resembles some naturally occurring polyphenolic flavones, such as fisetin and quercetin, with reported SIRT1-activating properties (Chart 2).

Chart 2. Putative SIRT1 Activators

has been controversy whether resveratrol, another polyphenolic SIRT1 activator, directly activates SIRT1 or not. It has been shown that in vitro, resveratrol activates SIRT1-mediated deacetylation of substrates that have a fluorophore covalently attached but not substrates lacking this fluorophore. To confirm that the detected SIRT2 inhibition by 1a was not caused by interaction with an artificial fluorophore, we further verified SIRT2 inhibition with two different methods. First, a Western blot analysis of the SIRT2-mediated deacetylation of acetylated α-tubulin was carried out and inhibition of the SIRT2-catalyzed reaction by 1a was observed (Figure 1A). Second, a SIRT2 activity assay based on the release of radioactive 14C-nicotinamide was performed in the presence of an acetylated peptidesubstrate (RSTGKK(Ac)APRKQ) without a fluorophore (Figure 1B). In this assay 1a gave 66% inhibition. Taken together, 1a was able to inhibit the deacetylation of three different substrates: an artificial substrate with a fluorophore and a peptide and a protein substrate without a fluorophore. On the basis of these results, a series of analogues of 1a was synthesized and evaluated as SIRT2 inhibitors.

Chemistry. The synthetic pathways toward the test compounds 1a–p, 3a,b, and 4–6 are presented in Scheme 1. The chroman-4-ones 1a–p were synthesized according to a procedure previously reported by our group. Commercially available 2'-hydroxyacetophenones were reacted with appropriate aldehydes in a base-promoted crossed aldol condensation followed by an intramolecular oxo-Michael addition. The reactions were conducted by heating ethanolic mixtures to 160–170 °C using microwave (MW) irradiation for 1 h in the presence of DIPA as base. The desired 2-alkyl-chroman-4-ones (1a–p) were isolated in low to high yields (17–88%). The outcome of the reaction was strongly depended on the substitution pattern of the acetophenones. In general, electron-deficient 2'-hydroxyacetophenones gave high yields of the desired chroman-4-ones whereas electron-donating groups led to higher amounts of byproduct originating from self-condensation of the used aldehyde. This caused purification problems, lowering the yields; for example, the 6,8-dimethyl- and 6-methoxy-substituted 2-pentylchroman-4-one derivatives 1d and 1h were obtained in only 17% yield. The enantiomers of 1a were separated from the racemic mixture by preparative HPLC on a chiral stationary phase. For the synthesis of the 2-pentylchromone 3a, chroman-4-one 1a was converted to the corresponding 3-bromo-2-pentylchroman-4-one 2 using Py·Br3 as the brominating agent. Interestingly, 2 was isolated as a diastereomeric mixture of 80:20 according to 1H NMR spectroscopy with the cis-isomer as the major product. Computational studies confirmed the higher stability of the cis-product. Hydrobromide elimination of 2 by CaCO3 in DMF in a microwave-assisted reaction provided the desired chromone 3a in 84% yield. Flavone 3b was prepared from 3'-bromo-5'-chloro-2'-hydroxyacetophenone via esterification with benzoyl chloride followed by a Baker–Venkataraman rearrangement yielding a diketo intermediate that was cyclized in an acid-catalyzed reaction. To obtain compounds 5 and 6, the carbonyl group in 1a was reduced by NaBH4 in MeOH, providing 8-bromo-6-chloro-2-pentylchroman-4-ol 4 in almost quantitative yield and in 96:4 diastereomeric ratio. With the chroman-4-ol 4 in hand, the hydroxy group could be removed either by dehydroxylation or dehydration. Dehydroxylation was carried out using triethylsilane as hydrogen source in the presence of BF3·Et2O yielding 8-bromo-6-chloro-2-pentylchroman 5 in moderate yield (44%). Dehydration was performed using a catalytic amount of p-toluenesulfonic acid with MgSO4 present as drying agent. The corresponding 2H-chromene 6 could be isolated in a yield of 63%.

Structure–Activity Relationship (SAR) Studies. The set of synthesized substituted chroman-4-one and chromone derivatives was used to explore the SAR (Table 1). The inhibitory activities of the synthesized compounds 1a–p, 3a,b, and 4–6 were determined using in vitro SIRT1, SIRT2, and SIRT3 assays. It is noteworthy that the most potent inhibitors (over 70% inhibition at 200 μM) are completely SIRT2 selective. They show less than 10% inhibition of SIRT1 and SIRT3 at 200 μM, the only exception being 16% inhibition of SIRT3 by compound 1m. The results from the SIRT2 assay are
summarized in Table 1 (see Supporting Information for results from the SIRT1 and SIRT3 assays).

In the initial SAR investigation the individual enantiomers of the lead compound 1a were tested. It turned out that the enantiomers had only slightly different inhibitory activities. Enantiomer (−)-1a was a more potent inhibitor with an IC_{50} value of 1.5 μM compared to (+)-1a with an IC_{50} of 4.5 μM. The unsaturated analogue of 1a, chromone 3a, was

Figure 1. Inhibition of SIRT2-mediated deacetylation reactions by compound 1a. (A) Western blot analysis of the inhibition of SIRT2-mediated α-tubulin deacetylation by 1a. The concentration of 1a was 200 μM, and measurements were performed at 30 min and 1 h. (B) Inhibition by 1a of the SIRT2-mediated deacetylation of the acetylated peptide RSTGGK(Ac)APRKQ. The reaction was detected by formation of the reaction product ^14C-nicotinamide.

Scheme 1. General Methods for the Syntheses of Compounds 1a−p, 2, 3a,b, and 4−6

Reagents and conditions: (a) appropriate aldehyde, DIPA, EtOH, MW, 160−170 °C, 1 h, 17−88%; (b) Py·Br, CH₂Cl₂, room temp, 2.5 h, 81%; cis/trans ratio 80:20; (c) CaCO₃, DMF, MW, 100 °C, 20 min, 84%; (d) i. benzoyl chloride, pyridine, room temp, 2 h; ii. KOH, pyridine, 50 °C, 4 h; iii. HCl, AcOH, reflux, 14 h, 89% (over three steps); (e) NaBH₄, MeOH/THF, 0 °C−rt, 15 min, 98%, 95:5 ratio of diastereomers; (f) Et₃SiH, BF₃·Et₂O, CH₂Cl₂, −78 °C−rt, 19 h, 44%; (g) p-TSA (cat.), MgSO₄, toluene, 90 °C, 1.5 h, 63%.

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The unsubstituted 2-pentylchroman-4-one (1a) showed strong inhibition. Replacement of the halogens with methyl substituents resulted in an IC50 value (1.5 ± 0.4 μM) identical to that of the unsubstituted 2-pentylchroman-4-one (1a). This supports the previous finding that larger substituents in the 6- and 8- positions are necessary to achieve any inhibition. To reveal whether the effect of variations of the substituents in the aromatic system is caused by steric or electrostatic properties, various disubstituted 2-pentylchroman-4-ones (1c–e) were synthesized. The change of the 6-chloro substituent in 1a to the larger but less electronegative 6-bromo substituent in 1c was tolerated and resulted in an IC50 value (1.5 μM) identical to that of the (−)-1a enantiomer. Interestingly, the difluorinated 1e with smaller but more electronegative substituents was considerably less active than the other dihalogenated derivatives (1a and 1c) but more potent than the unsubstituted 1b. This suggests that electron-withdrawing groups in general enhance activity but that electrostatic properties are not exclusively responsible for strong inhibition. Replacement of the halogens with methyl groups (1d) caused a slight decrease in activity compared to the chloro- and bromo-substituted 1a and 1c but a clear increase in activity compared to the difluorinated 1e. This supports the previous finding that larger substituents in the 6- and 8- positions are necessary to achieve significant inhibition. Altogether these results revealed that the size of the 6- and 8-substituents is important although electron-withdrawing properties can further improve the inhibitory activity.

To further probe the importance of the 6- and 8-substituents for inhibitory potency, derivatives lacking one of these groups were synthesized (1f–i). Compound 1f that only contains the 6-chloro substituent showed a decrease in activity. No change in activity was observed when the 6-chloro substituent was replaced with an electron-withdrawing nitro group (1g). Interestingly, with an electron-donating methoxy group in the same position (1h) the inhibitory activity decreased to 20% (the inhibitory activity was determined at a concentration of 200 μM). This particular example shows that the activity can be dramatically altered by the electronic nature of the substituent. Compound 1i lacking a substituent in the 6-position was significantly less potent than the lead compound 1a. Thus, the substituent in the 6-position is more important for activity than that in the 8-position. It was also clarified that electron-rich chroman-4-ones generally are less potent inhibitors than electron-poor compounds. One example of substitution in the 7-position was explored with the fluoro-substituted 1j which showed only weak inhibitory activity (18%).

The influence of the 2-substituent is reflected by compounds 1k–p and 3b in which the length and branching of the alkyl chain have been altered and also aromatic groups of different sizes have been introduced. It was shown that the inhibitory effect is dependent on the length of the alkyl chain. Chroman-4-one 1k with an n-propyl chain gave slightly better activity (76%, IC50 10.6 μM) than the n-heptyl substituted 1l (57%). However, the originally chosen pentyl group had the most optimal length among the studied alkyl derivatives. Branching of the chain in the vicinity of the chroman-4-one ring system insignificantly less active than rac-1a with an IC50 value of 5.5 μM.

The inactivity is dependent on the length of the alkyl chain. Chroman-4-one 1a with an IC50 value of 1.5 μM resulted in an IC50 value (1.5 ± 0.4 μM) identical to that of the unsubstituted 2-pentylchroman-4-one (1a). This supports the previous finding that larger substituents in the 6- and 8- positions are necessary to achieve any inhibition. To reveal whether the effect of variations of the substituents in the aromatic system is caused by steric or electrostatic properties, various disubstituted 2-pentylchroman-4-ones (1c–e) were synthesized. The change of the 6-chloro substituent in 1a to the larger but less electronegative 6-bromo substituent in 1c was tolerated and resulted in an IC50 value (1.5 μM) identical to that of the (−)-1a enantiomer. Interestingly, the difluorinated 1e with smaller but more electronegative substituents was considerably less active than the other dihalogenated derivatives (1a and 1c) but more potent than the unsubstituted 1b. This suggests that electron-withdrawing groups in general enhance activity but that electrostatic properties are not exclusively responsible for strong inhibition. Replacement of the halogens with methyl groups (1d) caused a slight decrease in activity compared to the chloro- and bromo-substituted 1a and 1c but a clear increase in activity compared to the difluorinated 1e. This supports the previous finding that larger substituents in the 6- and 8- positions are necessary to achieve significant inhibition. Altogether these results revealed that the size of the 6- and 8-substituents is important although electron-withdrawing properties can further improve the inhibitory activity.

Table 1. Results from Evaluation of Compounds 1a–p, 3a,b, and 4–6 in a SIRT2 Activity Assay

| Compd | R2                  | R3                  | R4                  | Inhibition ± SD at 200 μM (%) | IC50 (μM) | n.d. |
|-------|---------------------|---------------------|---------------------|-----------------------------|-----------|------|
| 1a    | (CH2)4CH3           | Cl                  | H                   | 88 ± 0.9                    | 4.3 (3.5–5.4) |      |
| (+)−1a| (CH2)4CH3           | Cl                  | H                   | 70 ± 0.8                    | 4.5 (3.5–5.9) |      |
| (−)−1a| (CH2)4CH3           | Cl                  | H                   | 91 ± 0.8                    | 1.5 (1.3–1.7) |      |
| 1b    | (CH2)4CH3           | H                   | H                   | 49 ± 4.8                    | n.d.      |      |
| 1c    | (CH2)4CH3           | Br                  | H                   | 92 ± 1.2                    | 1.5 (1.3–1.7) |      |
| 1d    | (CH2)4CH3           | CH3                 | H                   | 83 ± 0.7                    | 6.2 (4.7–8.1) |      |
| 1e    | (CH2)4CH3           | F                   | H                   | 30 ± 1.3                    | n.d.      |      |
| 1f    | (CH2)4CH3           | Cl                  | H                   | 55 ± 2.4                    | n.d.      |      |
| 1g    | (CH2)4CH3           | NO2                 | H                   | 58 ± 0.7                    | n.d.      |      |
| 1h    | (CH2)4CH3           | OCH3                | H                   | 20 ± 4.1                    | n.d.      |      |
| 1i    | (CH2)4CH3           | H                   | Br                  | 28 ± 1.1                    | n.d.      |      |
| 1j    | (CH2)4CH3           | H                   | F                   | 18 ± 1.0                    | n.d.      |      |
| 1k    | (CH2)4CH3           | Cl                  | Br                  | 76 ± 1.8                    | 10.6 (9.0–12.5) |      |
| 1l    | (CH2)4CH3           | Cl                  | H                   | 57 ± 2.5                    | n.d.      |      |
| 1m    | CH2CH2Ph            | Cl                  | H                   | 81 ± 0.7                    | 6.8 (5.8–8.0) |      |
| 1n    | CH(CH2)2            | Cl                  | H                   | 52 ± 1.0                    | n.d.      |      |
| 1o    | CH2CH3(3-indolyl)   | Cl                  | H                   | 53 ± 1.7                    | n.d.      |      |
| 1p    | CH2CH3(N/Ts)(3-indolyl) | Cl                  | H                   | 27 ± 1.6                    | n.d.      |      |
| 3a    | (CH2)4CH3           | Cl                  | H                   | 82 ± 0.4                    | 5.5 (4.8–6.2) |      |
| 3b    | Ph                  | Cl                  | H                   | 20 ± 1.4                    | n.d.      |      |
| 4     | (CH2)4CH3           | Cl                  | H                   | 31 ± 3.0                    | n.d.      |      |
| 5     | (CH2)4CH3           | Cl                  | H                   | 38 ± 1.3                    | n.d.      |      |
| 6     | (CH2)4CH3           | Cl                  | H                   | 38 ± 1.2                    | n.d.      |      |

SD, standard deviation (n = 3). IC50 (95% confidence interval). IC50 values were determined for compounds showing >70% inhibition of SIRT2 at 200 μM concentration. n.d. = not determined.
decreased the inhibitory activity; the isopropyl analogue 1n was less active (52%) than the n-propyl derivative 1k (76%, IC₅₀ 10.6 μM). Introduction of a phenyl group as in flavone 3b resulted also in decreased inhibition (20%) compared to the n-pentyl-substituted chromone 3a (82%, IC₅₀ 5.5 μM). These results showed that bulky groups directly connected to the ring system diminish the inhibitory effect against SIRT2. However, the phenethyl-substituted derivative 1m gave 81% inhibition (IC₅₀ 6.8 μM), which showed that substituents other than aliphatic chains are allowed if a spacer is introduced between the scaffold and the aromatic ring. Replacement of the phenyl group in 1m with sterically more demanding indole groups resulted in decreased activity. Compound 1o with an unsubstituted indole showed 53% inhibitory activity and was more active than the N-tosyl-substituted 1p (27% inhibitory activity). These results indicate a space limitation for the substituents in the 2-position and showed that more bulky groups in this position lower the activity considerably.

Finally, the impact of the carbonyl group was investigated and is demonstrated by compounds 4–6. Any modification of the carbonyl group resulted in a significant loss of inhibitory activity compared to 1a. Interestingly, a simple manipulation as a reduction of the ketone to a hydroxyl moiety (4) resulted in the same decrease of activity as the removal of the group (5 and 6). The lack of activity of 4–6 could be due to that an important hydrogen bonding interaction could no longer be formed with the enzyme. In case of 4, the low activity is likely to be due to conformational changes in the ring system resulting in changes in the orientation of the oxygen atom. These results showed that the presence of the carbonyl group is an essential feature to attain potent inhibitors.

We can conclude that there is an exceptionally close relationship between the molecular structure of the compounds and their SIRT2 inhibitory activity because even minor alterations in one of the four positions in the chroman-4-one ring system markedly affect the activity of the compounds.

**Determination of the Absolute Configuration of the Enantiomers of 1a.** The enantiomers of the lead compound 1a were separated by preparative chiral HPLC. The absolute configuration was intended to be determined by means of X-ray crystallography. Unfortunately, all attempts to obtain useful crystals of the enantiomers failed. A valid alternative for the determination of the absolute configuration of small molecules is to compare experimental and calculated vibrational circular dichroism (VCD) spectra. To predict the VCD spectra of low energy conformers of a molecule, density functional theory (DFT) calculations can be used. Highly flexible groups in a molecule lead to many conformers which have to be considered in the calculation of the VCD data. Therefore, to facilitate the configurational determination, the calculations were done on a slightly truncated structure (2-ethyl instead of 2-pentyl, Figure 2), as the change in the alkyl group is not expected to alter the VCD spectra to any greater extent. The experimental spectra of the two enantiomers of 1a were compared with the calculated spectra of the R- and the S-enantiomers of the modified structure.

As can be seen in the VCD spectra (Figure 3), several bands in the frequency region from 1500 to 1100 cm⁻¹ show good alignment between the experimental spectrum of (−)-1a and the calculated spectrum of the S-enantiomer of the 2-ethyl analogue. Equally good alignment in the same region is obtained when comparing the experimental spectrum of (+)-1a and the calculated spectrum of the R-enantiomer. Thus, from this study it is concluded that (−)-1a most likely has the S-configuration and (+)-1a the R-configuration.

**CONCLUSIONS**

A series of compounds based on the chromone and chroman-4-one scaffolds have been synthesized using an efficient one-step procedure. The derivatives were selective and potent SIRT2 inhibitors with IC₅₀ values in the low micromolar range. Among the investigated modifications, it was found that an alkyl chain with three to five carbons in the 2-position, larger, electron-withdrawing groups in the 6- and 8-positions, and an intact carbonyl group were crucial for high potency. Synthesis and evaluation of additional chroman-4-one analogues is ongoing in our laboratory. Special emphasis is directed toward the introduction of functional groups that can enhance the polarity of the derivatives.

**EXPERIMENTAL SECTION**

**General.** All reactions were carried out using magnetic stirring under ambient atmosphere if not otherwise noted. Room temperature corresponds to a temperature interval from 20 to 21 °C. All starting materials and reagents were obtained from commercial producers and were used without prior purification. Solvents were generally used as supplied by the manufacturer. Microwave reactions were carried out using a Biotage Initiator Sixty with fixed hold time modus in 2–5 mL or 10–20 mL capped microwave vials. All reactions were monitored by thin-layer chromatography (TLC) on silica-plated aluminum sheets (Silica gel 60 F254, E. Merck). Spots were detected by UV (254 or 365 nm). Purification by flash column chromatography was performed using an automatic Biotage SP4 Flash+ instrument. Prefabricated columns of three different cartridge sizes (surface area 500 m²/g, porosity 60 Å, particle size 40–63 μm) were used. The NMR spectra were measured with a JEOL JNM-ECP 400 or a Varian 400-400 MHz spectrometer. ¹H and ¹³C NMR spectra were measured at 400 and 100 MHz, respectively. Chemical shifts are reported in ppm with the solvent residual peak as internal standard [CDCl₃, δ(7.26), CDCl₄, δ(77.16)]. All NMR experiments were measured at ambient temperature. VCD spectra were recorded with a ChiralIR-2X from BioTools. The optical rotation was measured with an automatic polarimeter AA-S (Optical Activity Ltd.). Melting points were measured with a Büchi B-545 melting point apparatus or a Mettler FP82 hot stage equipped with a FP80 temperature controller and are uncorrected. Positive ion mass spectra (ESI-MS) were acquired with an LCQ quadrupole ion trap mass spectrometer (Finnigan LTQ) equipped with an electro-spray ionization source or on a Perkin-Elmer API 150EX mass spectrometer. Combustion analyses for CHN were measured on a Thermo Quest CE Instruments EA 1110 CHNS-O elemental analyzer. The syntheses of compounds 1a, 1m–p, and 3b have previously been described by our group, and the characterization data were in agreement with those reported in the literature.

**General Procedure for Synthesis of Chroman-4-ones 1b–n.**

The appropriate aldehyde (1.1 equiv) and DIPA (1.1 equiv) were added to a 0.4 M solution of the appropriate 2-hydroxycacetophenone in EtOH. The mixture was heated by microwave irradiation at 160–170 °C for 1 h (fixed hold time, normal absorption), diluted with...
CH₂Cl₂ and washed with NaOH (aq, 10%), HCl (aq, 1 M), water, and finally brine. The organic phase was dried over MgSO₄ filtered, and concentrated under reduced pressure. Purification by flash column chromatography gave chroman-4-ones 1b–n.

2-Pentylchroman-4-one (1b). The title compound was synthesized according to the general procedure from 2-hydroxyacetophene (1.00 mL, 8.31 mmol), hexanal (1.12 mL, 9.11 mmol), and DIPA (1.28 mL, 9.13 mmol). The crude product was purified by flash chromatography using EtOAc:hexane (5%) as eluent to obtain 1b (0.99 g, 55%) as a pale yellow viscous liquid. ¹H NMR δ 7.85 (dd, J = 7.8, 1.7, 0.4 Hz, 1H), 7.44 (dd, J = 8.3, 7.2, 1.8, 1H), 7.02–6.91 (m, 2H), 4.49–4.35 (m, 1H), 2.72–2.60 (m, 2H), 1.94–1.78 (m, 1H), 1.78–1.62 (m, 1H), 1.61–1.18 (m, 6H), 0.90 (t, J = 7.0 Hz, 3H). ¹³C NMR δ 192.7, 161.7, 135.9, 126.9, 121.1, 121.0, 117.9, 77.9, 43.0, 34.9, 31.6, 24.6, 22.5, 14.0. Anal. (C₉H₁₆O₃) C, H, N.

6,8-Dibromo-2-pentylchroman-4-one (1c). The title compound was synthesized according to the general procedure from 3',5'-dibromo-2-hydroxyacetophenone (0.29 g, 1.10 mmol), hexanal (0.14 mL, 1.14 mmol), and DIPA (0.16 mL, 1.14 mmol). Purification by flash column chromatography EtOAc:hexane (2%) and recrystallization from hexane gave 1c (0.21 g, 56%) as an off-white solid. Mp 80–82 °C. ¹H NMR δ 7.94 (d, J = 2.4 Hz, 1H), 7.83 (d, J = 2.4 Hz, 1H), 4.54–4.47 (m, 1H), 2.78–2.66 (m, 2H), 1.99–1.89 (m, 1H), 1.78–1.43 (m, 3H), 1.41–1.31 (m, 4H), 0.93–0.89 (m, 3H). ¹³C NMR δ 190.5, 157.1, 140.9, 128.8, 122.8, 113.6, 113.0, 79.0, 42.3, 43.6, 31.5, 24.6, 22.5, 13.9. Anal. (C₁₅H₁₃Br₂O₂) C, H, N.

6,8-Dimethyl-2-pentylchroman-4-one (1d). The title compound was synthesized according to the general procedure from 3',5'-dimethyl-2-hydroxyacetophenone (0.49 g, 2.98 mmol), hexanal (1.35 mL, 10.98 mmol), and DIPA (1.05 mL, 7.49 mmol). The crude product was purified twice by flash column chromatography using EtOAc:hexane (5%) to afford 1d (0.12 g, 17%) as an off-white solid. Mp 35–37 °C. ¹H NMR δ 7.53–7.50 (m, 1H), 7.14–7.17 (m, 1H), 4.38 (app dq, J = 8.0, 4.7 Hz, 1H), 2.60–2.70 (m, 2H), 2.56 (s, 3H), 2.21 (s, 3H), 1.83–1.94 (m, 1H), 1.74–1.28 (m, 7H), 0.94–0.86 (m, 3H). ¹³C NMR δ 192.7, 161.8, 136.0, 127.0, 121.2, 121.1, 118.0, 78.0, 43.1, 35.0, 31.6, 24.6, 22.6, 14.1. Anal. (C₉H₁₆O₃) C, H, N.

6,8-Difluoro-2-pentylchroman-4-one (1e). The title compound was synthesized according to the general procedure from 3',5'-difluoro-2-hydroxyacetophenone (0.20 g, 1.16 mmol), hexanal (0.16 mL, 1.28 mmol), and DIPA (0.18 mL, 1.28 mmol). The crude product was purified by flash column chromatography using EtOAc:hexane (3–6%) to afford 1e (70 mg, 23%) as an off-white oil which solidified in the refrigerator. ¹H NMR δ 7.35 (dd, J = 8.0, 3.0, 1.8 Hz, 1H), 7.06 (ddd, J = 10.1, 8.0, 3.1 Hz, 1H), 4.49 (dd, J = 10.4, 7.4, 5.1, 1.1 Hz, 1H), 2.79–2.68 (m, 2H), 1.98–1.89 (m, 1H), 1.78–1.69 (m, 1H), 1.60–1.31 (m, 6H), 0.94–0.89 (m, 3H). ¹³C NMR δ 190.8 (dd, J = 3.3, 2.1 Hz), 155.7 (dd, J = 244.2, 9.7 Hz), 151.9 (dd, J = 252.6, 10.9 Hz), 147.0 (dd, J = 11.6, 2.8 Hz), 122.8 (dd, J = 7.4, 1.7 Hz) 110.9 (dd, J = 280, 21.4 Hz), 107.3 (dd, J = 230, 4.2 Hz), 79.3, 43.0 (d, J = 1.3 Hz), 34.8, 31.6, 24.6, 22.6, 14.1. Anal. (C₁₀H₁₆F₂O₂) C, H, N.

6-Chloro-2-pentylchroman-4-one (1f). The title compound was synthesized according to the general procedure from 5'-chloro-2-hydroxyacetophenone (0.17 g, 1.00 mmol), hexanal (0.14 mL, 1.14 mmol) and DIPA (0.16 mL, 1.14 mmol). Purifications by flash column chromatography using EtOAc:hexane (3%), EtOAc:hexane (1.5–1.8%) and finally THF:hexane (1%) gave 1f (0.13 g, 51%) as a white solid. Mp 42–44 °C. ¹H NMR δ 7.82 (d, J = 2.7 Hz, 1H), 7.40 (dd, J = 8.8, 2.7 Hz, 1H), 6.93 (d, J = 8.8 Hz, 1H), 4.46–4.39 (dd, J = 10.4, 7.4, 5.3, 5.3 Hz, 1H), 2.7–2.62 (m, 2H), 1.92–1.82 (m, 1H), 1.74–1.65 (m, 1H), 1.60–1.25 (m, 6H), 0.93–0.88 (m, 3H). ¹³C NMR δ 191.6, 160.3, 135.9, 126.8, 126.4, 121.9, 119.8, 42.8, 34.9, 31.7, 24.7, 22.7, 14.1. Anal. (C₁₅H₁₄ClO₃) C, H, N.

6-Nitro-2-pentylchroman-4-one (1g). The title compound was synthesized according to the general procedure from 2'-hydroxy-5'-nitroacetophenone (0.50 g, 2.76 mmol), hexanal (0.37 mL, 3.04 mmol), and DIPA (0.43 mL, 3.07 mmol). Flash column chromatography of the crude product using EtOAc:hexane (5%) gave 1g as a slightly yellow solid (0.42 g, 58%). Mp 81–83 °C. ¹H NMR δ 8.77 (d, J = 2.8 Hz, 1H), 8.32 (dd, J = 9.1, 2.8 Hz, 1H), 7.10 (d, J = 9.1 Hz,
H1), 4.88 (d, J = 5.8 Hz, 0.2H), 4.40 (d, J = 1.7 Hz, 0.8H), 4.21 (ddd, J = 8.0, 5.4, 1.7 Hz, 0.8H), 2.16 (m, 0.8H), 1.84–1.76 (m, 1.2H), 1.67–1.29 (m, 6H), 0.95–0.88 (m, 3H). 13C NMR δ 184.4, 183.9, 155.7, 139.3, 139.0, 127.7, 126.9, 126.5, 126.0, 119.6, 112.9, 112.6, 82.7, 79.4, 49.4, 48.4, 32.4, 31.6, 31.3, 31.0, 24.9, 24.2, 22.43, 13.87.

8-Bromo-6-chloro-2-pentylchroman-4-one (3a). Chroman-4-one 2 (0.18 g, 0.43 mmol) was dissolved in DMP (3 mL), and CaCO3 (0.13 g, 1.30 mmol) was added. The mixture was heated by microwave irradiation at 100 °C for 20 min. The mixture was diluted with EtOAc, filtered, and washed with water. Finally, the filtrate was dried over Na2SO4, and concentrated under vacuum. Purification by flash column chromatography using EtOAc:hexane (5%) gave 3a (0.12 g, 84%) as a white solid. Ms 73–75 °C. 1H NMR δ 8.08 (d, J = 2.4 Hz, 1H), 7.83 (d, J = 2.4 Hz, 1H), 6.19 (s, 1H), 2.67 (t, J = 7.6 Hz, 2H), 1.85–1.70 (m, 2H), 1.45–1.31 (m, 4H), 0.91 (t, J = 6.8 Hz, 3H). 13C NMR δ 176.5, 170.5, 151.8, 136.6, 131.1, 125.5, 124.7, 112.7, 109.5, 34.2, 31.2, 26.4, 22.4, 14.0. Anal. (C16H18BrClO) C, H, N.

8-Bromo-6-chloro-2-pentylchroman-4-ol (4). A solution of 1a (0.40 g, 1.2 mmol) in MeOH:THF (20:5 mL) was cooled to 0 °C with an ice bath, and NaBH4 (55 mg, 1.5 mmol) was added. The mixture was allowed to warm to room temperature and stirred for 15 min. Water was added, and the aqueous phase was separated and extracted three times with EtOAc. The combined organic phases were washed with water, dried over MgSO4, filtered, and concentrated under reduced pressure. Chroman-4-ol (0.39 g, 98%) was obtained as a white solid consisting of a 96:4 mixture of diastereomers. 1H NMR δ 7.42–7.39 (m, 2H), 4.95–4.85 (m, 0.96H), 4.76–4.71 (m, 0.04H), 4.22–4.11 (m, 1H), 2.32 (ddd, J = 13.0, 6.2, 1.9 Hz, 1H), 1.90–1.33 (m, 9H), 0.94–0.89 (m, 3H). 13C NMR δ 150.2, 132.1, 128.5, 126.3, 125.5, 111.1, 77.5, 77.2, 76.7, 76.2, 65.7, 37.6, 35.4, 31.7, 24.9, 22.7, 14.1. Anal. (C16H18BrClO) C, H, N.

8-Bromo-6-chloro-2-pentylchroman-4-yl (5). A solution of chroman-4-ol 4 (0.20 g, 0.60 mmol) in CH2Cl2 (10 mL) was cooled to −78 °C. Triethylamine (1.4 mL, 8.76 mmol) was added dropwise, followed by boron trifluoride etherate (0.3 mL, 2.4 mmol), and the mixture was stirred for 19 h at room temperature. The mixture was filtered, and the filtrate was concentrated under reduced pressure. Purification by flash column chromatography using heptane gave 5 (0.64 mg, 44%) as a white solid. Ms 33–36 °C. 1H NMR δ 7.32 (d, J = 2.5 Hz, 1H), 6.97 (d, J = 2.6 Hz, 1H), 4.09–4.01 (m, 1H), 2.87–2.69 (m, 2H), 2.04–1.96 (m, 1H), 1.85–1.29 (m, 9H), 0.94–0.89 (m, 3H). 13C NMR δ 150.6, 130.4, 128.4, 124.7, 112.7, 111.5, 77.5, 77.2, 76.2, 76.5, 65.7, 37.6, 35.4, 31.7, 24.9, 22.7. 14.1. Anal. (C16H18BrClO) C, H, N.

8-Bromo-6-chloro-2-pentyl-2H-chromene (6). Chroman-4-ol 4 (0.13 g, 0.39 mmol) was dissolved in toluene (20 mL) and heated to 90 °C for 1.5 h in the presence of a catalytic amount of p-toluenesulfonic acid and anhydrous MgSO4 (50 mg). The mixture was filtered, and the filtrate was concentrated under reduced pressure. Purification by flash column chromatography using EtOAc:heptane (5%) gave 6 (0.12 g, 94%) as a slightly yellow oil which solidified in the refrigerator. 1H NMR δ 7.30 (d, J = 2.5 Hz, 1H), 6.88 (d, J = 2.5 Hz, 1H), 6.29 (dd, J = 9.9, 1.6 Hz, 1H), 5.78 (dd, J = 9.9, 3.6 Hz, 1H), 4.98–4.92 (m, 1H), 1.84–1.73 (m, 1H), 1.67–1.24 (m, 7H), 0.92–0.88 (m, 3H). 13C NMR δ 148.8, 131.5, 128.0, 125.8, 125.1, 124.0, 111.4, 76.4, 35.2, 31.5, 24.5, 22.5, 14.0. Anal. Calc for C16H17BrClO: C, 53.2; H, 5.11. Found: C, 53.2; H, 5.05.

Biochemistry. In Vitro Fluo de Lys Assay for SIRT1, SIRT2, and SIRT3 Activities. The Fluo de Lys fluorescence assays were based on the method described in the BioMol product sheet (Enzo Life Sciences) using the BioMol KI177 substrate for SIRT1 and the KI179 substrate for SIRT2 and SIRT3. The determined Kₐ value for SIRT1 for KI177 was 58 µM, and the Kₐ for SIRT2 for KI179 was 198 µM. The Kₐ for SIRT3 for KI179 was reported by BioMol to be 32 µM. The Kₐ values for SIRT1, SIRT2 and SIRT3 for NAD⁺ were reported by BioMol to be 558 µM, 547 µM, and 2 mM, respectively.

Briefly, assays were carried out using the Fluo de Lys acetylated peptide substrate at a concentration corresponding to 0.7 Kₐ and NAD⁺ (N6522, Sigma) at a concentration corresponding to 0.9 Kₐ, recombinant GST-SIRT1/2-enzyme or recombinant His-SIRT3 and
SIRT assay buffer (HDAC assay buffer, K1143, supplemented with 1 mg/mL BSA, A3803, Sigma). GST-SIRT1 and GST-SIRT2 were produced as described previously.43,44 His-SIRT3 (BML-SE270) was purchased from Enzo Life Sciences. The buffer, Fluor de Lys acetylated peptide substrate, NAD\(^+\), and DMSO/compounds in DMSO (2.5 \(\mu\)L in 50 \(\mu\)L total reaction volume; DMSO from Sigma, D2650) were preincubated for 5 min at room temperature. The reaction was started by adding the enzyme. The reaction mixture was incubated for 1 h at 37 °C. After that, Fluor de Lys developer (KI176) and nicotinamide (2 mM in HDAC assay buffer giving total volume of 50 \(\mu\)L) were added, and the incubation was continued for 45 min at 37 °C. Fluorescence readings were obtained using a VictorTM 1420 Multilabel Counter (Wallac, Finland) with excitation wavelength 355 nm and emission 460 nm.

The IC\(_{50}\) values were based on nine-point dose–response determinations (2000 \(\mu\)M, 1000 \(\mu\)M, 100 \(\mu\)M, 10 \(\mu\)M, 1 \(\mu\)M, 0.1 \(\mu\)M, 0.01 \(\mu\)M, 0.001 \(\mu\)M, and 0.0001 \(\mu\)M) where more dose points were added between the critical concentrations depending on the compound. Each experiment was repeated at least three times, and values were calculated using the Graph Pad Prism Software version 4.03 (19922005 GraphPad Software, Inc.).

In Vitro \(^{14}\)C-NAD\(^+\) Assay for SIRT2 Activity. Inhibition of SIRT2 by compound 1a was verified by a protocol based on release of \(^{14}\)C-nicotinamide from NAD\(^+\) during the reaction.45 Radioactive nicotinamide was detected using a TLC-based technique.46 The assay was carried out using [carboxyl-\(^{14}\)C]-NAD\(^+\) (Amersham Pharmacia, CAA372), acetylated RSTGG/K(Ac)APRKQ peptide (196 \(\mu\)M) (purchased from Caslo Laboratory ApS), and SIRT assay buffer and DMSO (0.7 \(\mu\)L) or compound 1a in DMSO. The final reaction concentration of 1a was 200 \(\mu\)M. The total reaction volume was 9.7 \(\mu\)L and reactions were started by adding GST-SIRT2.44,46 The reaction mixture was incubated for 1 h at 37 °C. The entire volume of the reaction mixture was spotted on a TLC plate (Whatman, PE SIL G). Plates were eluted with ethanol/ammonium acetate (2.5 M) (80:20), dried, and exposed overnight to a medical X-ray film (SuperRX-film, Fujifilm Corporation, Tokyo, Japan), developed with a Kodak X-OMAT 2000 developing machine (Eastman Kodak Company, Rochester, NY) and scanned with an Epson Perfection V750 Pro scanner (Epson America Inc., Long Beach, CA) using the Jasc Paint Shop Pro 8 software (Jasc Software Inc., Eden Prairie, MN). Images were processed with the use of the UN-SCAN-IT gel software (Silk Scientific Corporation, Orem, UT).

Western Blot Analysis for Acetylated \(\alpha\)-Tubulin. Western blot analysis was carried out as described previously.37 Briefly, acetylated \(\alpha\)-tubulin (0.5 \(\mu\)g) isolated from rat stem cells was incubated 30 or 60 min at 37 °C (total volume of reaction mixture 50 \(\mu\)L) with recombinant GST-SIRT2 enzyme (3 \(\mu\)L), NAD\(^+\) (500 \(\mu\)M), SIRT assay buffer (41 \(\mu\)L), and DMSO (2.5 \(\mu\)L) or 1a in DMSO. The final reaction concentration of 1a was 200 \(\mu\)M. The GST-SIRT2 enzyme was prepared as described previously.43,44 Samples were solubilized in NuPAGE LDS sample buffer (NP0007, Invitrogen, Carlsbad, CA), boiled for 4 min, resolved at 200 V on a 10% SDS-PAGE gel, and electrophoretically transferred to an ECL-nitrocellulose membrane (Amersham, GE Healthcare, London, UK). Blots were blocked for 1 h with nonfat dry milk (3%) in PBS (phosphate-buffered saline) and Tween-20 (0.05%). Membranes were probed for 2 h at room temperature with monoclonal antiacetylated tubulin antibody (Clone 6-11B-1, Sigma, T 6793, 1:8000), washed, and incubated for 2 h with horseradish peroxidase-conjugated donkey-antimouse (1:50000, Amersham, NA9340V) secondary antibody.

Results were detected by Immobilon Western Chemiluminescent HRP Substrate (ECL-kit, Millipore, Billerica, MA). Filters were exposed to a medical X-ray film (SuperRX-film), developed using a Kodak X-OMAT 2000 developing machine, and scanned with the Epson Perfection V750 Pro scanner using the Jasc Paint Shop Pro 8 software. Images were processed with the use of the UN-SCAN-IT gel software.

**Determination of Absolute Configuration of (\(+\)) and (\(-\)) 1a** Using VCD. Experimental: Each sample was prepared by dissolving 12 mg of the solid material in 150 \(\mu\)L of CDCl\(_3\). Subsequently, the solutions were transferred to a 0.75 mm BaF\(_2\) cell, and the VCD spectra for each enantiomer were acquired for 20 h for (\(+\)) 1a and 15 h for (\(-\)) 1a in a BioTools ChiralIR-2X instrument equipped with a single photoelastic modulator. The resolution was 4 cm\(^{-1}\). DFT calculations: A Monte Carlo molecular mechanics search for low energy geometries was conducted for both enantiomers of the truncated structure using MacroModel within the Maestro graphical interface (Schrodinger Inc.). All conformers within 5 kcal/mol of the lowest energy conformer were used as starting points for DFT minimizations within Gaussian09. Optimized structures and VCD spectra were determined for each conformer. In these calculations, the B3LYP generalized gradient approximation (GGA) exchange-correlation density functional was used. The 6-31G\(^*\) basis set was used.

**ASSOCIATED CONTENT**

### Supporting Information

Synthetic procedures (1a, 1n–p, 3b), characterization data and elemental analysis for all tested compounds, complete list of SIRT1–3 activity assay results, separation of enantiomers, IR spectrum of 1a, and \(^1\)H NMR and \(^{13}\)C NMR spectra of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

**AUTHOR INFORMATION**

*Corresponding Author*

*(K.L.) Tel: +46 31 786 9031; fax: +46 31 772 1394; e-mail: luthman@chem.gu.se. (E.M.J.) Tel: +358 40 355 2460; fax: +358 17 162424; e-mail: Elina.jarho@uef.fi.

**Author Contributions**

These authors contributed equally to this work.

**Notes**

The authors declare no competing financial interest.

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**ABBREVIATION USED**

BSA, bovine serum albumin; DFT, density functional theory; DIPA, N,N-diisopropylamine; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; ECL\(_h\), enhanced chemiluminescence; ESI-MS, electrospray ionization mass spectrometry; GST, glutathione-S-transferase; HDAC, histone deacetylase; HPLC, high performance liquid chromatography; HRP, horseradish peroxidase; IC\(_{50}\) the half maximal inhibitory concentration; KHMD\(_s\), potassium hexamethyldisilazane; LDS, lithium dodecyl sulfate; MW, microwave; NAD\(^+\), nicotinamide adenine dinucleotide; NMR, nuclear magnetic resonance; PBS, phosphate-buffered saline; Py, pyridine; SAR, structure–activity relationship; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SIRT, silent information regulator human type; THF, tetrahydrofuran; TLC, thin layer chromatography; p-TSA, p-toluene sulfonic acid; UV, ultraviolet; VCD, vibrational circular dichroism
REFERENCES

(1) Imai, S.; Armstrong, C. M.; Kaerbelein, M.; Guarente, L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 2000, 403, 795–800.

(2) Sauve, A. A. Sirtuin chemical mechanisms. Biochim. Biophys. Acta 2010, 1804, 1591–1603.

(3) Guarente, L.; Nakagawa, T. Sirtuins at a glance. J. Cell. Sci. 2011, 124, 833–840.

(4) Taylor, D. M.; Maxwell, M. M.; Luthi-Carter, R.; Kazantsev, A. G. Biological and Potential Therapeutic Roles of Sirtuin Deacetylases. Cell. Mol. Life Sci. 2008, 65, 4000–4018.

(5) Sinclair, D.; Michan, S. Sirtuins in mammals: insights into their biochemical function. Biochem. J. 2007, 404, 1–13.

(6) Guarente, L. Sirtuins, Aging, and Medicine. N. Engl. J. Med. 2011, 364, 2235–2244.

(7) Luthi-Carter, R.; Taylor, D. M.; Pallos, J.; Lambert, E.; Amore, A.; Parker, A.; Moffitt, H.; Smith, D. L.; Runne, H.; Goke, O.; Kuhn, A.; Xiang, Z. M.; Maxwell, M. M.; Reeves, S. A.; Bates, G. P.; Neri, C.; Thompson, L. M.; Marsh, J. L.; Kazantsev, A. G. SIRT2 inhibition achieves neuroprotection by decreasing sterol biosynthesis. Proc. Natl. Acad. Sci. U.S.A. 2010, 107, 7927–7932.

(8) Kazantsev, A.; Outeiro, T. F.; Marques, O. Therapeutic role of sirtuins in neurodegenerative disease. Biochim. Biophys. Acta 2008, 1782, 363–369.

(9) Superti-Furga, G.; Huber, K. After the grape rush: Sirtuins as epigenetic drug targets in neurodegenerative disorders. Bioorg. Med. Chem. 2011, 19, 3616–3624.

(10) Zhu, X. W.; Bonda, D. J.; Lee, H. G.; Camins, A.; Pallas, M.; Casadesus, G.; Smith, M. A. The sirtuin pathway in ageing and Alzheimer disease: mechanistic and therapeutic considerations. Lancet Neurol. 2011, 10, 275–279.

(11) Guarente, L.; Donmez, G.; Wang, D.; Cohen, D. E. SIRT1 Suppresses beta-amylloid Production by Inactivating the alpha-Secretase Gene ADAM10. Cell 2010, 142, 320–332.

(12) Milno, J. C.; Denu, J. M. The Sirtuin family: therapeutic targets to treat diseases of aging. Curr. Opin. Chem. Biol. 2008, 12, 11–17.

(13) Dryden, S. C.; Nahhas, F. A.; Nowak, J. E.; Goustin, A. S.; Tainsky, M. A. Role for human SIRT2 NAD-dependent deacetylase activity in control of mitotic exit in the cell cycle. Mol. Cell. Biol. 2003, 23, 3173–3185.

(14) Inoue, T.; Hiratsuka, M.; Osaki, M.; Oshimura, M. The molecular biology of mammalian SIRT proteins· SIRT2 in cell cycle regulation. Cell Cycle 2007, 6, 1011–1018.

(15) North, B. J.; Marshall, B. L.; Borra, M. T.; Denu, J. M.; Verdin, E. The human Sir2 ortholog, SIRT2, is an NAD(+) dependent tubulin deacetylase. Mol. Cell 2003, 11, 347–344.

(16) Pavletich, N. P.; Finnin, M. S.; Donijian, J. R. Structure of the histone deacetylase SIRT2. Nat. Struct. Biol. 2001, 8, 621–625.

(17) Schemes, J.; Sippel, W.; Jung, M. Histone deacetylase inhibitors that target tubulin. Cancer Lett. 2009, 222, 223–232.

(18) Garske, A. L.; Smith, B. C.; Denu, J. M. Linking SIRT2 to Parkinson’s disease. ACS Chem. Biol. 2007, 2, 529–532.

(19) Outeiro, T. F.; Kontopoulos, E.; Altmann, S. M.; Kufareva, I.; Grigor, M.; Lifou, E.; Chung, P.; Kiselevski, A.; Zhang, L. L.; Scherer, B.; Sinclair, D. A. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature 2003, 425, 191–196.

(20) Borra, M. T.; Smith, B. C.; Denu, J. M. Mechanism of human SIRT1 activation by resveratrol. J. Biol. Chem. 2005, 280, 17187–17195.

(21) Pacholec, M.; Bleasdale, J. E.; Chunryk, B.; Cunningham, D.; Flynn, D.; Garofalo, R. S.; Griffith, D.; Grigor, M.; Loulakis, P.; Pabst, B.; Qiu, X. Y.; Stockman, D.; Thanabal, V.; Varghese, A.; Ward, J.; Withka, J.; Ahn, K. SIRT1720, SIRT2183, SRT1460, and Resveratrol Are Not Direct Activators of SIRT1. J. Biol. Chem. 2010, 285, 8340–8351.

(22) First eluting enantiomer: [α]D +82.5 (c = 0.2, hexane, 23 °C), second eluting enantiomer: [α]D −92.5 (c = 0.2, hexane, 23 °C).

(23) Reddy, N. J.; Sharma, T. C. Reduction of 3-bromoflavonones. Bull. Chem. Soc. Jpn. 1979, 52, 647–648.

(24) Compound 2 has not been tested, as the present study is focusing on changes in the substitution pattern in the aromatic ring and the 2-position of the chroman-4-one scaffold.

(25) Freedman, T. B.; Cao, X. L.; Dukor, K. R.; Nafie, L. A. Absolute configuration determination of chiral molecules in the solution state using vibrational circular dichroism. Chirality 2003, 15, 743–758.

(26) Stephens, P. J.; Devlin, F. J.; Pan, J. J. The determination of the absolute configurations of chiral molecules using vibrational circular dichroism (VCD) spectroscopy. Chirality 2008, 20, 643–663.

(27) Kiviranta, P. H.; Suuronen, T.; Wallén, E. A. A.; Leppänen, J.; Tervonen, J.; Kyrölenko, S.; Salminen, A.; Poso, A.; Jarho, E. M. N-Ethacoyl-Lysine-Containing Tri-, Tetra-, and Pentapeptides as SIRT1 and SIRT2 Inhibitors. J. Med. Chem. 2009, 52, 2153–2156.

(28) Kiviranta, P. H.; Leppänen, J.; Rinne, V. M.; Suuronen, T.; Kyrölenko, O.; Kyrölenko, S.; Kuusisto, E.; Tervo, A. J.; Järvinen, T.; Salminen, A.; Poso, A.; Wallén, E. A. A. N-(3-(4-hydroxyphenyl)-


doi.org/10.1021/jm0005288j J. Med. Chem. 2012, 55, 7104–7113
propenoyl)-amino acid tryptamides as SIRT2 inhibitors. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2448−2451.

(44) Tervo, A. J.; Kyrylenko, S.; Niskanen, P.; Salminen, A.; Leppänen, J.; Nyronen, T. H.; Järvinen, T.; Poso, A. An in silico approach to discovering novel inhibitors of human sirtuin type 2. *J. Med. Chem.* **2004**, *47*, 6292−6298.

(45) McDonagh, T.; Hixon, J.; DiStefano, P. S.; Curtis, R.; Napper, A. D. Microplate filtration assay for nicotinamide release from NAD using a boronic acid resin. *Methods* **2005**, *36*, 346−350.

(46) Tanny, J. C.; Moazed, D. Coupling of histone deacetylation to NAD breakdown by the yeast silencing protein Sir2: Evidence for acetyl transfer from substrate to an NAD breakdown product. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 415−420.

(47) Huhtiniemi, T.; Suuronen, T.; Rinne, V. M.; Wittekindt, C.; Lahtela-Kakkonen, M.; Jarho, E.; Wallén, E. A. A.; Salminen, A.; Poso, A.; Leppänen, J. Oxadiazole-carbonylaminothioureas as SIRT1 and SIRT2 inhibitors. *J. Med. Chem.* **2008**, *51*, 4377−4380.