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N-(3-(4-Hydroxyphenyl)-propenoyl)-amino acid tryptamides as SIRT2 inhibitors

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Abstract—A series of *N*-(3-(4-hydroxyphenyl)-propenoyl)-amino acid tryptamides was based on a previously reported new SIRT2 inhibitor from our group, and it was designed to study if the molecular size of the compound could be reduced. The most potent compounds, *N*-(3-(4-hydroxyphenyl)-propenoyl)-2-aminoisobutyric acid tryptamide and *N*-(3-(4-hydroxyphenyl)-propenoyl)-L-alanine tryptamide, were equipotent, 30% smaller in molecular weight, and slightly more selective (SIRT2/SIRT1) than the parent compound.

Silent information regulator human type 2 (SIRT2) enzyme belongs to the class III histone deacetylase (HDAC) protein family.[1,2] SIRT2, which is located principally in the cytoplasm of brain and muscle cells,[3] is one of the seven human sirtuin type homologues.[1,4] The class III proteins are nicotinamide adenine dinucleotide (NAD^+) dependent proteins, from which SIRT2 has been shown to catalyze the deacetylation of an acetylated lysine-40 of α-tubulin, resulting in free nicotinamide and 2′- and 3′-*O*-acetyl-ADP-ribose.[1,5–7] The Sir2 protein family have been connected with several cellular functions, for example, chromatin silencing, cell cycle, metabolism, and life span.[1,8–10] It has been shown that SIRT2 is a mitotic checkpoint protein[11] and it interacts with the homeobox transcription factor, HOXA10.[12] It has been postulated that inhibitors of SIRT2 might be beneficial in the treatment of cancer and neurodegenerative diseases.[13,14]

The NAD^+-dependent deacetylase activity is inhibited by nicotinamide. A few other SIRT2 inhibitors have also been reported. A3 and sirtinol were the first potent SIRT2 inhibitors.[15] 1,4-Bis-[2-(4-hydroxy-phenyl)-ethylamino]-anthraquinone,[16] *para*-sirtinol,[17] EX-527,[18] cambinol,[13] and Ro 31-8220[19] have been reported recently.

Based on our previous results[16,20] and examinations of favorable interactions between the known SIRT2 inhibitors and the putative binding site, a receptor-based virtual screening was carried out. Compound **1** (Tripos 360702) was the most potent compound that was found

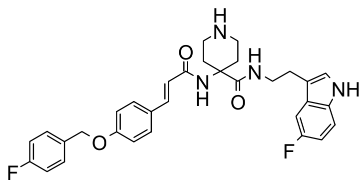

Figure 1. Compound **1** found by modeling and virtual screening.[21]

Keywords: SIRT2; HDAC; Inhibitor.

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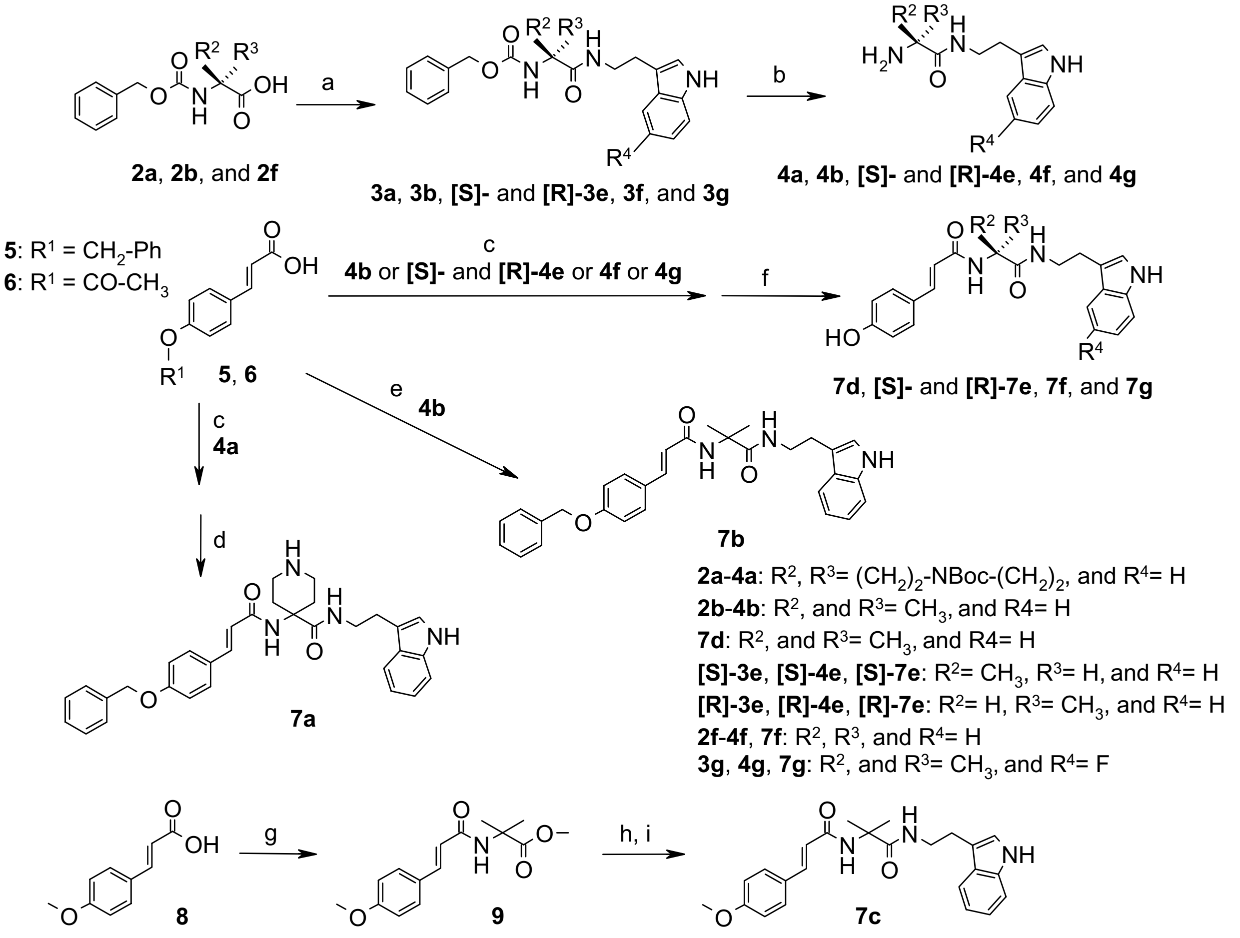
in this study and it had an IC_{50} of 51 μM (Fig. 1).[21] Compound **1** had a structural scaffold that was new for SIRT2 inhibitors. The desire to reduce the molecular size of compound **1** was the main focus for the present study. The design of the new series of compounds was based on simplifying the structure and finding the essential parts of **1** for the inhibitory activity.

The synthetic routes are presented in Scheme 1. The starting material 4-amino-1-Boc-piperidine-4-carboxylic acid was synthesized as described in the literature.[22] The amino groups of the amino acids **2a**, **2b**, and **2f** were protected with benzyl chloroformate. The carboxylic acid was activated with ethyl chloroformate[23] or DCC[24] and reacted with tryptamine. *N*-Cbz-L-alanine *N*-hydroxysuccinimide ester and *N*-Cbz-D-alanine *N*-hydroxysuccinimide ester were reacted with tryptamine in THF.[25] The Cbz group was removed with palladium (10%) on activated charcoal and either ammoniumformate or hydrogen gas in methanol. 3-(4-Benzyloxyphenyl)-propenoic acid (**5**) was synthesized from 3-(4-hydroxyphenyl)-propenoic acid and benzyl bromide. 3-(4-Acetoxyphenyl)-propenoic acid (**6**) was synthesized by protecting the phenolic hydroxyl group with acetic anhydride. Compound **7a** was synthesized from **5** with ethyl chloroformate activation followed by reaction with **4a**.[23] The removal of the Boc group yielded compound **7a**. To form compound **7b**, compound **5** was reacted with oxalyl chloride to form the acid chloride which was reacted with 2-amino-isobutyric acid tryptamide (**4b**) in DCM. Compounds **7d**, [*S*]-**7e**, [*R*]-**7e**, and **7g** were also synthesized from **6** with ethyl chloroformate activation followed by reaction with the appropriate amino acid tryptamide.[23] Compound **7f** was synthesized from compound **6** with DCC activation followed by reaction with glycine tryptamide (**4f**). The acetyl protection group was hydrolyzed with K_2CO_3 in water and methanol or CH_3ONa in methanol, yielding compounds **7d**–**7g**. In most amide formation reactions ethyl chloroformate activation was found to be the most successful method. The yields of the amide bond formations varied between 14% and 93%.

Another synthetic procedure was used for compound **7c**. 3-(4-Methoxyphenyl)-propenoic acid (**8**) was syn-



Scheme 1. Reagents and conditions: (a) 1—Et_3N, ethyl chloroformate, DCM or THF, -20 or 0 °C, 2—Et_3N, tryptamine; or DMAP, tryptamine, DCC, $CHCl_3$, 0 °C to reflux (*N*-Cbz-L-alanine *N*-hydroxysuccinimide ester and *N*-Cbz-D-alanine *N*-hydroxysuccinimide ester were reacted with tryptamine in THF, yielding compounds [*S*]- and [*R*]-**3e**.); (b) ammoniumformate, 10% Pd/C, MeOH; or H_2, 10% Pd/C, MeOH; (c) 1—Et_3N, ethyl chloroformate, THF or DCM, -20 or 0 °C, 2—Et_3N, **4a** or **4b** or [*S*]-**4e** or [*R*]-**4e** or **4g**; or DMAP, **4f**, DCC, DCM, and 1,4-dioxane, 0 °C; (d) TFA, anisole, sodium thiophenolate, DCM, 0 °C; (e) 1—oxalyl chloride, DMF, DCM; 2—**4b**, Et_3N, DCM; (f) K_2CO_3, H_2O, MeOH, 0 °C; or 1M CH_3ONa in MeOH, DCM, 0 °C; (g) 1—Et_3N, pivaloyl chloride, DCM, 0–25 °C, 2—Et_3N, 2-amino-isobutyric acid methyl ester; (h) $LiOH·H_2O$, H_2O, MeOH; (i) 1—Et_3N, ethyl chloroformate, DCM, -20 °C, 2—Et_3N, tryptamine.

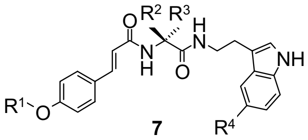
thesized as described in the literature.[26] Compound **8** was activated with pivaloyl chloride and reacted with 2-amino-isobutyric acid methyl ester. The methyl ester of the obtained product **9** was hydrolyzed with $LiOH \cdot H_2O$ in water and methanol. The obtained free carboxylic acid was activated with ethyl chloroformate and reacted with tryptamine to yield compound **7c**. This synthetic procedure was not useful when 3-(4-hydroxyphenyl)-propenoic acid was used because the removal of the methyl ester would also have removed the acetyl protection group of the phenolic hydroxyl group. EX-527 was used as a reference compound. EX-527 was synthesized as described in the literature.[18]

The compounds and their inhibitory activities are presented in Table 1. The inhibitory activities were tested in a Fluor de Lys fluorescence-based assay. Poor water solubility of some compounds was observed when determining the inhibition at higher concentrations. This was the main reason not to determine the IC_{50} values of the compounds with less than 50% inhibition at 200 μM. The importance of the fluorine atoms for the inhibitory activity of **1** was investigated with **7a**. Removal of the fluorine atoms did not have a significant effect on the inhibitory activity. Opening the piperidine ring and removing the amine functionality in the side-chain by using a 2-aminoisobutyric acid group in the middle of the structure resulted in **7b**, with a significantly lower inhibitory activity, inhibition of 32.6% at 200 μM. Furthermore, removal of the phenyl group resulted in **7c**, which was slightly more potent, with an inhibition of 55.4% at 200 μM and an IC_{50} of 99 μM. The structure was further simplified by removing the methyl group from the methoxy group, resulting in **7d**. Compound **7d** was equipotent with **1** and it showed that the chemical structure could be reduced in size without affecting the inhibitory activity for SIRT2. The molecular weight of **1** was reduced about 30%. Replacing the aminoisobutyric acid group by an L-alanine group gave [*S*]-**7e**, which had an inhibition of 88.6% at 200 μM and an IC_{50} of 47 μM. Compound [*S*]-**7e** showed also selectivity for SIRT2. However, replacing the aminoisobutyric acid group by a D-alanine group gave [*R*]-**7e**, with an inhibition of 8.5% at 200 μM. The structure was further simplified by replacing the alanine group by a glycine group resulting in **7f**, which was less potent than a L-alanine group but more potent than a D-alanine group. Finally, the effect of the fluorine atom on the indole ring was studied with **7g**. Compound **7g** showed that the fluorine atom does not have a positive effect on the inhibitory activity since **7g** had a slightly lower inhibitory activity compared to **7d**.

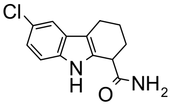
In addition, the selectivity for SIRT2 was studied (Table 1). As earlier reported, SIRT2 inhibitors are also good SIRT1 inhibitors. Interestingly, compound **7d** showed a slightly higher selectivity for SIRT2 than **1**. At the concentration of 200 μM compound **1** inhibits SIRT1 94.1% but **7d** only 6.6%. Compound [*S*]-**7e** is almost as selective as **7d**.

In conclusion, the essential parts of compound **1** for the inhibitory activity were identified, and the study showed that the molecular weight of compound **1** could be reduced 30% while maintaining the inhibitory activity. In addition, the most potent compounds **7d** and [*S*]-**7e** were slightly more selective for SIRT2 (SIRT2/SIRT1) than compound **1**.

Table 1. Compounds and their inhibitory activities for SIRT2 and SIRT1 (95% confidence intervals for IC_{50} given in parentheses)



7

Compound	R1	R2	R3	R4	Inhibition at 200 μM ± SD,[a] (%) SIRT2	Inhibition at 200 μM ± SD,[a] (%) SIRT1	IC_{50} (μmol/L) SIRT2[b]	IC_{50} (μmol/L) SIRT1[b]
1	CH_2-Ph-F	$(CH_2)_2$–NH–$(CH_2)_2$		F	77.3 ± 4.8	94.1 ± 3.5	51 (27–75)	73 (47–114)
7a	CH_2–Ph	$(CH_2)_2$–NH–$(CH_2)_2$		H	79.1 ± 1.4	97.0 ± 1.9	63 (41–96)	52 (38–70)
7b	CH_2–Ph	CH_3	CH_3	H	32.6 ± 18.2	10.2 ± 6.4	—	—
7c	CH_3	CH_3	CH_3	H	55.4 ± 3.3	10.3 ± 4.3	99 (66–150)	—
7d	H	CH_3	CH_3	H	83.3 ± 3.7	6.6 ± 1.8	50 (23–109)	—
[*S*]-**7e**	H	CH_3	H	H	88.6 ± 0.8	17.7 ± 1.0	47 (28–79)	—
[*R*]-**7e**	H	H	CH_3	H	8.5 ± 6.0	3.2 ± 5.5	—	—
7f	H	H	H	H	44.3 ± 10.6	9.9 ± 1.2	—	—
7g	H	CH_3	CH_3	F	67.5 ± 1.1	7.0 ± 4.0	80 (53–120)	—
10[18] EX-527					89.4 ± 2.8	98.9 ± 0.3	14 (8–25)	0.28 (0.23–0.34)

[a] SD, standard deviation.

[b] IC_{50} were determined for compounds which had over 50% inhibition at 200 μM for SIRT2 or SIRT1.

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Supplementary data

Detailed experimental procedures for the syntheses, NMR spectra, ESI-MS results, and elemental analysis data for the new compounds, and procedures obtaining human SIRT1 and SIRT2 recombinant proteins, and in vitro assay for SIRT2 activity are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.02.023.

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