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Evaluation of benzoic acid derivatives as sirtuin inhibitors

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ABSTRACT

Employing a genetically modified yeast strain as a screening tool, 4-dimethylaminobenzoic acid (**5**) was isolated from the marine sediment-derived *Streptomyces* sp. CP27-53 as a weak yeast sirtuin (Sir2p) inhibitor. Using this compound as a scaffold, a series of disubstituted benzene derivatives were evaluated to elucidate the structure activity relationships for Sir2p inhibition. The results suggested that 4-alkyl or 4-alkylaminobenzoic acid is the key structure motif for Sir2p inhibitory activity. The most potent Sir2p inhibitor, 4-*tert*-butylbenzoic acid (**20**), among the tested compounds in this study turned out to be a weak but selective SIRT1 inhibitor. The calculated binding free energies between the selected compounds and the catalytic domain of SIRT1 were well correlated to their measured SIRT1 inhibitory activities.

Sirtuins are a group of NAD^+-dependent histone deacetylases (HDACs) that are evolutionally conserved from bacteria to mammals.[1] This group of enzymes regulates the key biological functions including gene silencing and cell cycle by removing acetyl groups from lysine residues in the histones or non-histone substrates. To date, seven human isoforms (SIRT1–7) have been found and categorized as class III HDACs that are different from classical zinc-dependent class I/II/IV HDACs.[2] Recently, SIRT1 and SIRT2 have been considered as molecular targets for cancer chemotherapy. The physiological functions of SIRT1 with regard to tumorigenesis include the negative regulation of the tumor suppressor gene 53,[3] the positive regulation of the oncoprotein B-cell lymphoma 6 protein (BCL6),[4] and induction of the FOXO-1-dependent vascular growth factor-C (VEGF-C).[5] On the other hand, SIRT2 promotes cancer cell proliferation due to the enhanced stability of the Myc oncoproteins.[6] It has been reported that apoptosis caused by p53 accumulation in HeLa cells[7] and granulocytic differentiation in the acute promyelocytic leukemia (APL)[8] can be induced based on down-regulation and inhibition of SIRT2. Though SIRT1 and SIRT2 are also reported to show tumor-suppressing effects[9–11] and to be down-regulated in specific cancer types,[12] the tumor promoting effects listed above strongly suggest SIRT1/2 inhibitors have great potential to be anticancer drugs. Encouraging evidence includes significant cytotoxic properties of the two SIRT1/2 inhibitors, sirtinol (**1**: synthetic[13]) and toxoflavin (**2**: natural product[14]), against the MCF-7 and A549 cancer cell lines, respectively.[15,16] It is interesting that the potent and selective SIRT1 inhibitor, EX-527 (**3**: synthetic[17]), required at least 100 μM to effectively repress MCF-7 cell proliferation.[18] However, the selective SIRT2 inhibitor, AC-93253 (**4**: synthetic), exhibited potent cytotoxic effects (IC_{50} 10–100 nM) against the prostate (DU-145), lung (A549, NCI-H460) and pancreas (MiaPaCa2) cancer cell lines with a great therapeutic window (up to 200-fold).[19]

We have recently reported the HDAC screening method using the genetically modified yeast strain DMY2843 to identify SIRT1/2 inhibitors from marine-sediment derived actinomycetes.[20] The primary screening for the extract library using the yeast assay selected a total of 19 actinomycete strains that would produce yeast sirtuin (Sir2p) inhibitors. A new polyketide tetramic acid derivative designated as streptosetin A with weak Sir2p and SIRT1/2 inhibitory activities has been isolated from one of the active strains, *Streptomyces* sp. CP13-10.[20] Our continued search for sirtuin inhibitors from the remaining active strains led to the identification of 4-dimethylaminobenzoic acid (**5**) produced by the *Streptomyces* sp. CP27-53 strain as a Sir2p inhibitor. In this article, we report the identification of **5** and structure activity relationships (SARs) and SIRT inhibitory activities of benzoic acid derivatives and related analogues of **5**.

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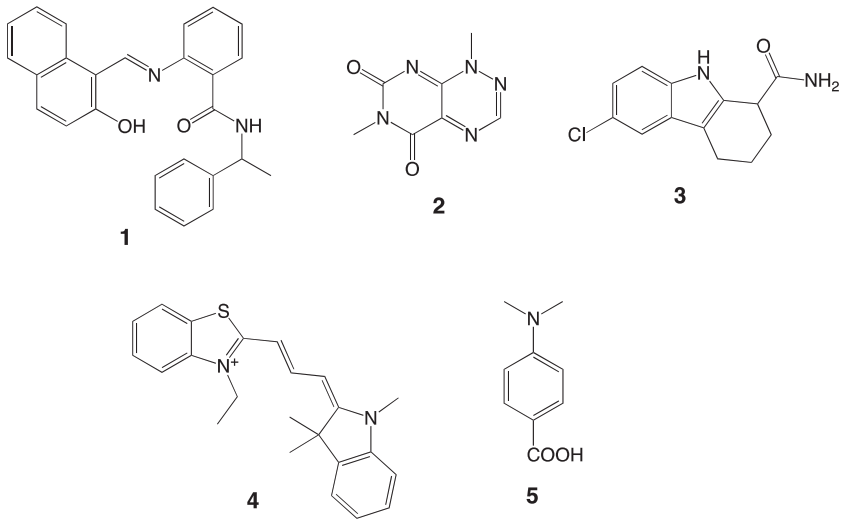

The marine sediment-derived *Streptomyces* sp. CP27-53 was cultured in a liquid medium (15 L) containing soluble starch (1%), yeast extract (0.4%), peptone (0.2%), $CaCO_3$ (0.1%) and $FeSO_4{\cdot}7H_2O$ (40 mg) in artificial seawater adjusted to pH 7.4 for 10 days at 30 °C at 200 rpm. The culture was separated to broth and pellet by centrifugation. The broth was treated with HP20 to absorb organic compounds, which were eluted with MeOH, whereas the pellet was extracted with MeOH three times. The combined MeOH extract was cleaned by liquid–liquid partition between EtOAc and H_2O to give an organic extract. Yeast screening of the HPLC peak library created from the organic extract revealed that the compound eluting at 14.3 min in the HPLC chromatogram was responsible for the Sir2p inhibitory activity (Fig. S1). Furthermore, this active compound was purified by reversed-phase HPLC and identified as 4-dimethylaminobenzoic acid (**5**) based on the spectroscopic data (see Supplementary data). The structure was further confirmed by direct comparison of the ^{1}H and ^{13}C NMR data with those of an authentic sample. This compound showed Sir2p inhibitory activity with an MIC of 200 μM after 48 h against the yeast strain.

To elucidate the SARs for Sir2p inhibition by **5**, a series of substituted benzoic acid derivatives and related analogues of **5** were evaluated using the yeast strain DMY2843 (Table 1 and Fig. 1). The compounds in Group A (**6**–**9**) were inactive against the yeast strain, which suggested that the two functional groups, dimethylamino group and carboxylic acid, must be on *para*-positions to show Sir2p inhibitory activity. The MIC values of 400 and 800 μM for compounds **10** and **11** in Group B, respectively, indicated that more methyl groups on the nitrogen enhanced the Sir2p

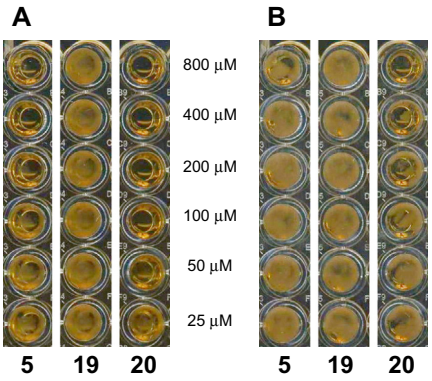

Figure 1. Yeast HDAC screening results (48 h) for **5**, **19** and **20**. (A) YPD media with 5-fluoro (5-FOA) and (B) YPD media. Sir2p inhibition was defined as selective activity when the tested compound resulted in the death of yeast cells (clear) only in the presence of 5-FOA. Cytotoxicity was observed as the death of yeast cells in both media A and B.

Table 1
Minimum inhibition concentration (MIC) values of disubstituted benzene derivatives based on Sir2p inhibition against the yeast strain DMY2844

Structural group		R^1	R^2	Location	MIC (μM)
A	**5**	$(CH_3)_2N$–	–COOH	*p*	200
	6	$(CH_3)_2N$–	–COOH	*m*	NA[a]
	7	$(CH_3)_2N$–	–COOH	*o*	NA
	8	$(CH_3)_2N$–	None		NA
	9	None	–COOH		NA
B	**10**	CH_3NH–	–COOH	*p*	400
	11	NH_2–	–COOH	*p*	800
C	**12**	$(CH_3)_2N$–	$-COOCH_3$	*p*	400
	13	$(CH_3)_2N$–	$-CONH_2$	*p*	NA
	14	$(CH_3)_2N$–	$-SO_3H$	*p*	NA
	15	$(CH_3)_2N$–	–Br	*p*	NA
	16	$(CH_3)_2N$–	$-NO_2$	*p*	NA
D	**17**	CH_3–	–COOH	*p*	800
	18	$(CH_3)_2CH$–	–COOH	*p*	200
E	**19**	$(CH_3)_3N^+$–	$-COO^-$	*p*	NA
	20	$(CH_3)_3C$–	–COOH	*p*	50

[a] Not active at 800 μM.