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Identification of novel SIRT2-selective inhibitors using a click chemistry approach

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ABSTRACT

A series of 114 SIRT inhibitor candidates was assembled using 'click chemistry', by reacting two alkynes bearing 2-anilinobenzamide pharmacophore with 57 azide building blocks in the presence of Cu(I) catalyst. Screening identified two SIRT2-selective inhibitors, which were more SIRT2-selective than AGK2, a known SIRT2 inhibitor. These findings will be useful for further development of SIRT2-selective inhibitors.

Sirtuins are a class of enzymes known as NAD^+-dependent protein deacetylases that regulate a variety of biological processes including aging, transcription and metabolism.[1–3] Humans have seven distinct sirtuin gene products (SIRT1-7).[4] Among the seven human sirtuins, SIRT2 is the only cytosolic protein. SIRT2 deacetylates nonhistone proteins in cytoplasm and is involved in various biological events. For example, SIRT2 destabilizes microtubules by deacetylating α-tubulin,[5] and deacetylation of phosphoenolpyruvate carboxykinase by SIRT2 regulates gluconeogenesis.[6] Although the functions of SIRT2 have not yet been fully understood, they have been suggested to be associated with certain disease states. It has been reported that inhibition of SIRT2 rescued α-synuclein toxicity and modified inclusion morphology in a cellular model of Parkinson's disease.[7] SIRT2 inhibition also has been reported to reduce neuronal cholesterol reduction and to be neuroprotective in cellular models of Huntington's disease.[8] Therefore, SIRT2-selective inhibitors are of interest not only as tools for elucidating in detail the biological functions of the enzyme, but can also be considered as potential therapeutic agents for neurodegenerative diseases.

Several classes of small molecule SIRT2-selective inhibitors have been identified so far (Fig. 1). These include AGK2 (**1**),[7] compounds **2**,[9] **3**[10] and **4**.[11] Previously, we identified 2-anilinobenzamide **5** as SIRT1-selective inhibitors.[12,13] The structure–activity relationship (SAR) studies of anilinobenzamide derivatives revealed that *meta*- or *para*-substituted 2-anilinobenzamides such as compounds **6** and **7** tend to show SIRT2 selectivity (Fig. 2).[14]

We and other groups previously described the identification of enzyme inhibitors from a triazole compound library generated by the use of Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC),[15–

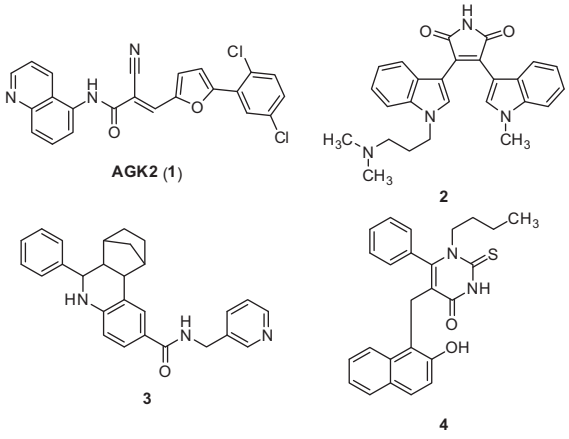

Figure 1. Reported small molecule SIRT2-selective inhibitors.

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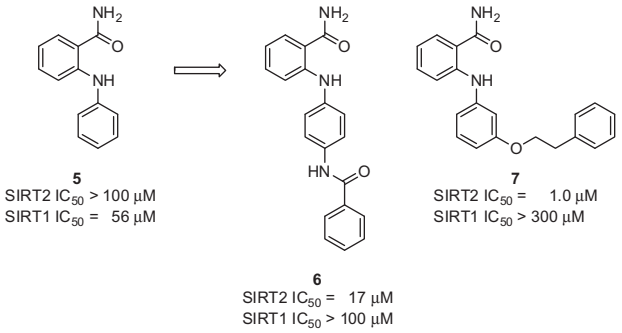


Figure 2. SIRT2-selective inhibitors discovered by our group.

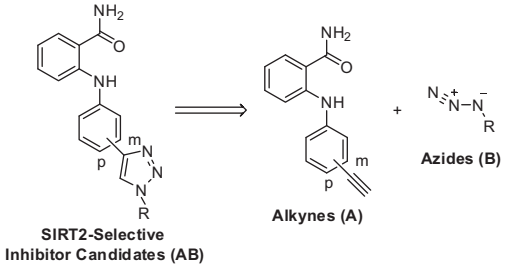
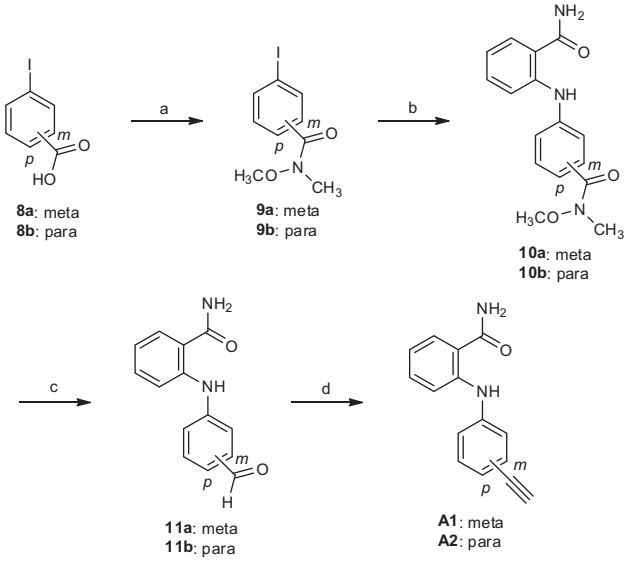


Figure 3. Design of triazole-containing SIRT2-selective inhibitors.



Scheme 1. Reagents and conditions: (a) (i) $SOCl_2$, CH_2Cl_2, reflux, (ii) *N*,*O*-dimethylhydroxylamine hydrochloride, Et_3N, CH_2Cl_2, rt, 91% for **9a**, 83% for **9b**; (b) 2-aminobenzamide, Pd_2dba_3, 2-dicyclohexylphosphino-2′,4′,6′-triisopropyl biphenyl, K_2CO_3, *tert*-BuOH, reflux, 15% for **10a**, 81% for **10b**; (c) $LiAlH_4$, THF, 0 °C, 45% for **11a**, q. y. for **11b**; (d) dimethyl 1-diazoacetonylphosphonate, MeOH, rt, 20% for **A1**, 22% for **A2**.

[17] a representative reaction in click chemistry.[18] Following these studies, we performed a further click chemistry approach, seeking to find novel SIRT2-selective inhibitors. We describe here the rapid identification of novel SIRT2-selective inhibitors via the use of click chemistry to generate a library of *meta*- and *para*-substituted 2-anilinobenzamides as SIRT inhibitor candidates.

Based on the previous SAR studies of anilinobenzamide derivatives,[14] we designed a library of candidates as SIRT inhibitors bearing a triazole group at the *meta* or *para* position (Fig. 3).

Accordingly, we prepared two alkynes **A1** and **A2** as shown in Scheme 1. Treatment of iodobenzoic acids **8** with thionyl chloride afforded the corresponding acid chlorides which were converted to Weinreb amides **9** by the reaction with *N*,*O*-dimethylhydroxylamine. Buchwald–Hartwig reaction between 2-aminobenzamide and compounds **9** using 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl as a ligand gave diphenylamine compounds **10**. Compounds **10** were allowed to react with lithium aluminum hydride at 0 °C to give aldehydes **11** and subsequent Seyferth–Gilbert homologation using dimethyl 1-diazoacetonylphosphonate yielded the desired alkynes **A1** and **A2**.

Next, the 114-member 2-anilinobenzamide library was assembled using click chemistry in microtiter plates. Each of the two alkynes A (1 equiv) was mixed with each of the 57 azides B (Fig. 4) (1.2 equiv), which were previously prepared by us,[16,17,19] in the presence of $CuSO_4$ (0.2 equiv), sodium ascorbate (1 equiv), and tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) (0.2 equiv) in a solvent mixture of DMSO/H_2O (1:1).[20] The disappearance of the starting materials and the quantitative formation of the triazole products were confirmed by TLC and LC–MS (HPLC purity of the resulting triazole and remaining azide: >95%).

These 114 triazole compounds could be screened for SIRT inhibitory activity without further purification. To find SIRT2-selective inhibitors, compounds **A1B1**–**A2B57** were initially tested for activity against SIRT2 at 10 μM.[21] In this SIRT2 assay, alkynes (10 μM) and azides (10 μM) used for this study and a mixture of $CuSO_4$ (2 μM), sodium ascorbate (10 μM) and TBTA (2 μM) were totally inactive. As shown in Figure 5, two hits emerged from the screen. Crude compounds **A1B11** and **A2B57** showed SIRT2-inhibitory activity comparable to AGK2 (**1**). Furthermore, at the concentration of 10 μM, crude **A1B11** and **A2B57** were totally inactive against SIRT1 and SIRT3 (data not shown), suggesting the SIRT2 selectivity of **A1B11** and **A2B57**.

Because **A1B11** and **A2B57** used in the primary screening were crude compounds, compounds **A1B11** and **A2B57** were resynthesized and purified. Scheme 2 illustrates the resynthesis of triazoles **A1B11** and **A2B57**. Cu(I)-catalyzed coupling of alkyne **A1** with **B11** and **A2** with **B57** afforded tirazoles **A1B11** and **A2B57**, respectively. The resynthesized compounds **A1B11** and **A2B57** were purified by column chromatography and recrystallization.[22]

The pure **A1B11** and **A2B57** were then examined for inhibitory effects on SIRT2, SIRT1 and SIRT3.[21] The results of the enzyme assays are shown in Table 1. In these assays, compounds **A1B11** and **A2B57** showed potent SIRT2-inhibitory activity with IC_{50}s of 5.3 and 6.3 μM, respectively. The SIRT2-inhibitory activity of **A1B11** and **A2B57** was comparable to that of AGK2 (**1**). Furthermore, while AGK2 (**1**) inhibited SIRT1 and SIRT3 with IC_{50}s of 30 and 91 μM, respectively, **A1B11** and **A2B57** did not inhibit either SIRT1 or SIRT3 at concentrations up to 100 μM, showing high selectivity for SIRT2 over both SIRT1 and SIRT3. Thus, **A1B11** and **A2B57** are more selective SIRT2 inhibitors than AGK2 (**1**) in these enzyme assays.

In summary, we have rapidly identified novel SIRT2-selective inhibitors by using click chemistry to generate libraries of candidate molecules. The advantage of this work over others is the rapidness of identifying novel SIRT2 inhibitors. It should be possible to obtain even more potent and selective SIRT2 inhibitors by means of further structural development. SIRT2-selective inhibitors are thought to have considerable potential both for the development of novel therapeutic agents and as tools for biological research. Detailed studies of the SIRT2-selective inhibitors and their analogues are under way.

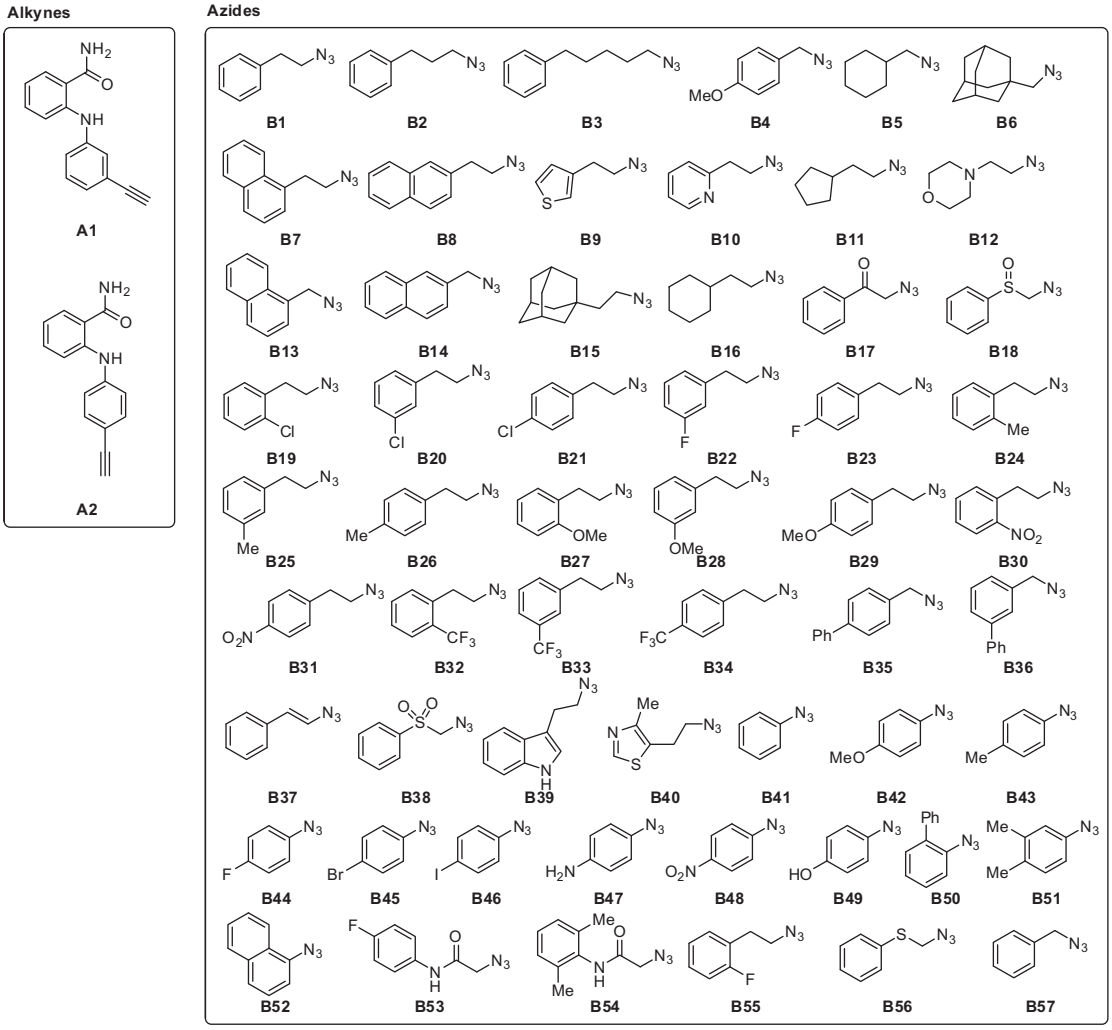


Figure 4. Structures of alkynes **A** and azides **B** used for this study.

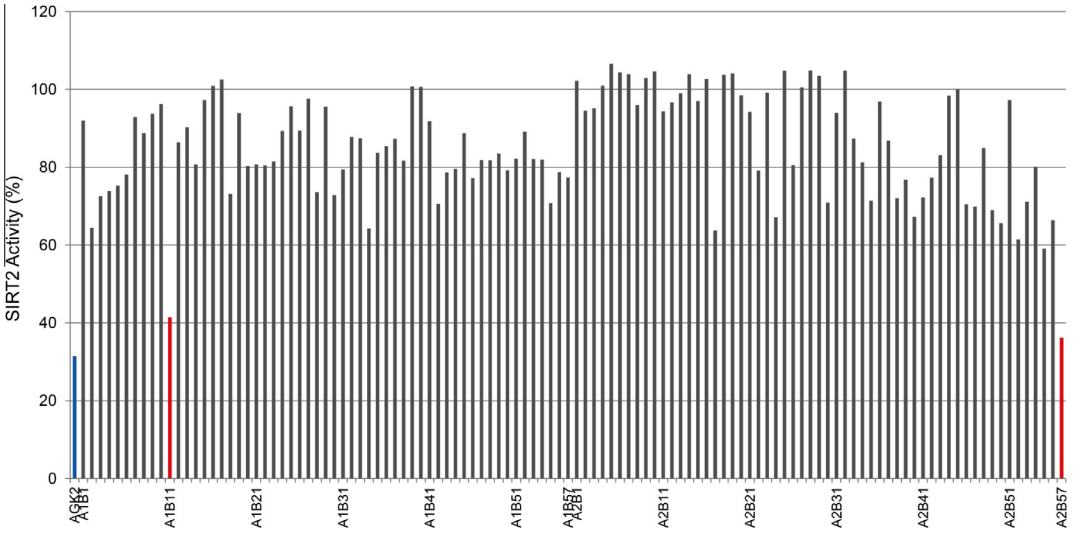
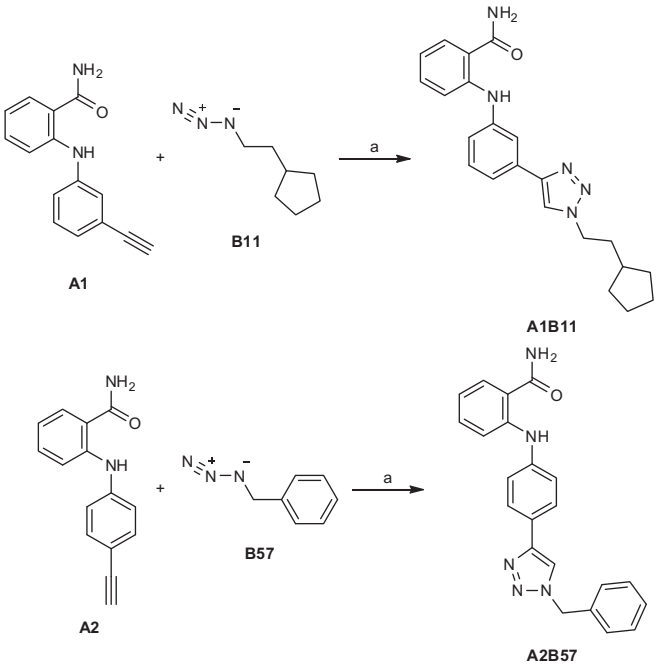


Figure 5. Activity of SIRT2 in the presence of 114 2-anilinobenzamides (10 μM).



Scheme 2. Reagents and conditions: (a) (i) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, TBTA, DMSO, rt; (ii) column chromatography; (iii) recrystallization, 20% for **A1B11**, 60% for **A2B57**.

Table 1
SIRT1-, SIRT2 and SIRT3-inhibitory activities of AGK2 (**1**), **A1B11** and **A2B57**[a]

Compound	IC_{50} (μM)		
	SIRT2	SIRT1	SIRT3
AGK2	3.5	30	91
A1B11	5.3	>100	>100
A2B57	6.3	>100	>100

[a] Values are the means of at least two experiments.

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20. *The triazole library was prepared using the following procedure:* To a solution of alkyne (25 mM, 20 μL), azide (30 mM, 20 μL), and TBTA (10 mM, 10 μL) in DMSO was added an aqueous solution of $CuSO_4{\cdot}5H_2O$ (4 mM, 25 μL) on a 96-well plate. To the resulting mixture was added an aqueous solution of sodium ascorbate (20 mM, 25 μL), and the mixture was shaken for 24 h at room temperature. Reactions were monitored by TLC and LCMS. After the reactions were completed, the triazoles were diluted to desired concentrations for enzyme assays by adding DMSO.
21. SIRT activity assay was performed using SIRT fluorimetric drug discovery kits (AK-555, AK-556, and AK-557, BIOMOL Research Laboratories), according to the supplier's protocol. SIRT (human, recombinant) (15 μL/well), NAD^+ (1 mM), and various concentrations of samples were incubated at 37° C for 60 min, and Fluor de Lys-SIRT substrate (25 μM) was added to the mixture. Reactions were stopped after 60 min by adding Fluor de Lys Developer II with nicotinamide, which stops further deacetylation. Then, 45 min after addition of this developer, the fluorescence of the wells was measured on a fluorometric reader with excitation set at 360 nm and emission detection set at 460 nm. The value of % inhibition was calculated from the fluorescence readings of inhibited wells relative to those of control wells. The compound concentration resulting in 50% inhibition was determined by plotting log[Inh] versus the logit function of% inhibition. IC_{50} values were determined by means of regression analysis of the concentration/inhibition data.
22. ***A1B11***: mp 56–61° C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 9.58 (1H, s), 7.73 (1H, s), 7.70 (1H, s), 7.52–7.47 (2H, m), 7.41–7.30 (4H, m), 7.29–7.20 (1H, m), 7.19 (1H, d, J = 8.1 Hz), 6.78 (1H, t, J = 7.0 Hz), 4.41 (2H, t, J = 7.5 Hz), 2.00–1.94 (2H, m), 1.81–1.77 (4H, m), 1.75–1.60 (2H, m), 1.69–1.20 (3H, m). MS (EI) m/z 389 (M^+). HRMS (EI) calcd for $C_{22}H_{19}N_5O$, 389.2216; found, 389.2216. Purity (HPLC) 98%.
A2B57: mp 172–176° C. ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 9.60 (1H, s), 7.74 (2H, d, J = 8.5 Hz), 7.61 (1H, s), 7.48 (1H, d, J = 8.0 Hz), 7.42–7.36 (4H, m), 7.33–7.29 (3H, m), 7.26–7.23 (2H, m), 6.78 (1H, t, J = 8.0 Hz), 5.58 (2H, s). MS (EI) m/z 369 (M^+). HRMS (EI) calcd for $C_{22}H_{19}N_5O$, 369.1590; found, 369.1591. Purity (HPLC) 97%.