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Search for a novel SIRT1 activator: Structural modification of SRT1720 and biological evaluation

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ABSTRACT

Syntheses and biological evaluation of novel SRT1720 derivatives are described in search for new candidates of SIRT1 activator. Several parts of the SRT1720 structure, including piperazine moiety, quinoxaline ring on the amide group, and position of the amide function, were modified, and the assay results indicated that transfer of the *ortho* amide-substituent regarding to the imidazo[1,2-*b*]thiazole core onto the *meta* position resulted in improvement of SIRT1 activation ability. Modeling analyses of SRT1720 and the most potent derivative bound to model complex of SIRT1 with peptide substrate were also performed.

Sirtuins are a family of NAD^+-dependent histone deacetylases (HDACs), and play significantly important roles with regard to metabolic pathway, apoptosis, inflammation, neuroprotection, cancer, and so on.[1] Among them (SIRT1–SIRT7), SIRT1 has been well studied because of beneficial effects of its modulators toward such human diseases.[2] For example, SIRT1 inhibitors have been one of potential anticancer therapeutic targets, and a number of small molecules possessing such effects have been reported so far.[3] On the other hand, exploration of SIRT1 activators have been rather limited, which are expected to have a high potential as novel drugs for age-related diseases treatment such as diabetes and metabolic disorders.[4]

As shown in Figure 1, resveratrol (**1**) is one of the most common SIRT1 activators, belonging to poly-phenolic compounds, and its biological profiles have been extensively studied and reviewed to date.[5] Mai et al. have reported that 1,4-dihydropyridine derivatives (**2**) exert an interesting SIRT1 modulating property, highly dependent on alkyl substituents on the N-1 position; *N*-benzyl derivatives behave as a potent SIRT1 activator whereas the other alkyl derivatives exhibit a SIRT1 inhibiting activity.[6] Artificial compounds having hetero-bicyclic scaffolds, including oxazolo[4,5-*b*]pyridines and imidazo[1,2-*b*]thiazoles, have been reported as a series of potent SIRT1 activators by Sirtris Pharmaceuticals.[7] Especially, SRT1720 (**3**) was discovered to be the most potent activating derivative and proved its beneficial effects on type-II diabetes model mice by in vivo studies.[7] Inspired by attractive potential of SRT1720 as a therapeutic agent for various disorders, we have also examined and reported the efficacy of SRT1720 on inflammation, tumor metastasis, and amelioration of fatty liver.[8]

Although in-depth SAR studies have been performed regarding various imidazo[1,2-*b*]thiazole derivatives,[7b] we have tried further exploration of novel SIRT1 activator based on the SRT1720 structure. In this Letter, we wish to disclose that transfer of the *ortho* amide-substituent regarding to the imidazo[1,2-*b*]thiazole core onto the *meta* position in the SRT1720 structure results in improvement of SIRT1 activating ability, which are assessed by means of in vitro assay system.

SRT1720 (**3**) was synthesized as a reference compound according to the reported procedure,[7b] which includes construction of the imidazo[1,2-*b*]thiazole nucleus (**4**) from 2-aminothiazole-4-carboxylate and α-bromoacetophenone derivative, incorporation of the piperazine unit (**5**), and quinoxaloyl-amide formation (Scheme 1). In the precedent report, [7b] a variety of SRT1720 derivatives having various aromatic rings instead of quinoxaline were synthesized and surveyed their SIRT1 activation. Here, we synthesized three new derivatives **6**–**8** from **5**, which have not been reported yet, via reduction of the nitro group followed by installation of the corresponding acyl groups by esterification (Scheme 1). In addition, the piperazine unit of SRT1720 was modified according to Scheme 2. The alcohol **4** was converted to MEM ether **9** and methyl ether **10**, and the quinoxaloyl-amide unit was

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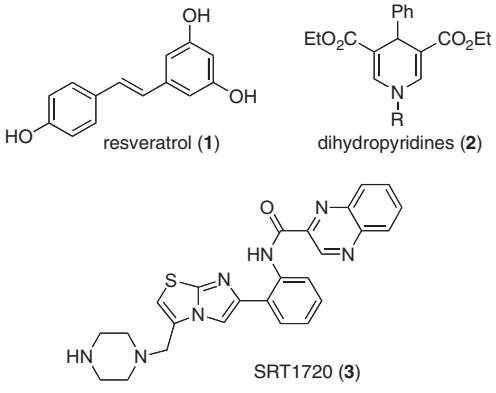
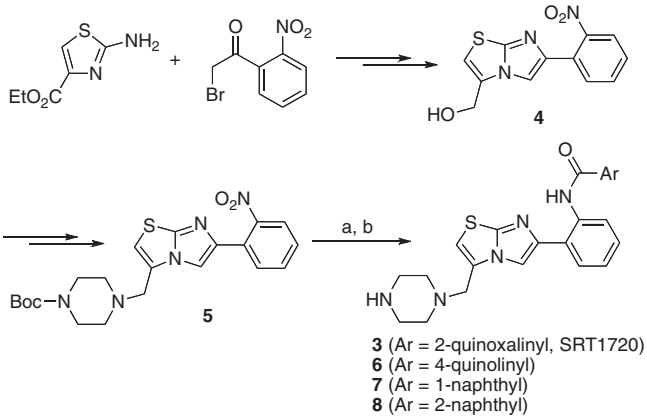
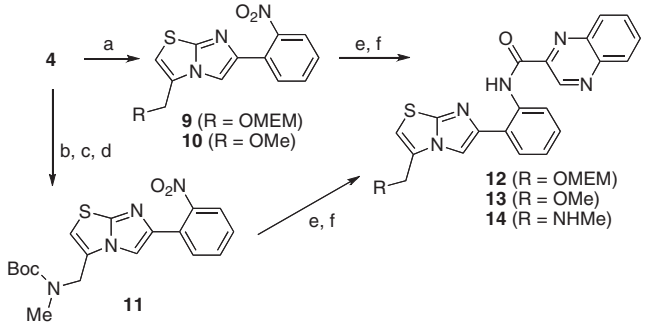


Figure 1. Reported SIRT1 activators.



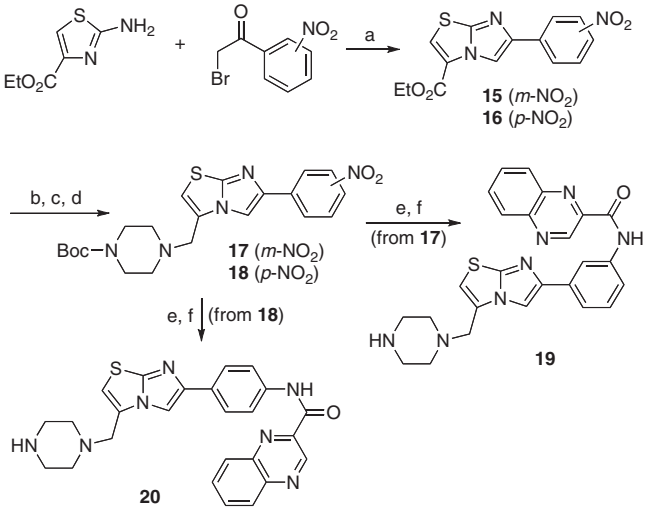
Scheme 1. Reagents and conditions: (a) NaSH, MeOH/H_2O, reflux, 5 d; (b) 4-quinolinecarboxylic acid, EDC, DMAP, CH_2Cl_2, rt, 1 h; then TFA, CH_2Cl_2, rt, 1 h (**6**: 60% from **5**), 1-naphthoyl chloride, pyridine, $CHCl_3$, rt, 2 h; then TFA, CH_2Cl_2, rt, 1 h (**7**: 63% from **5**), 2-naphthoyl chloride, pyridine, $CHCl_3$, rt, 3 h; then TFA, CH_2Cl_2, rt, 1 h (**8**: 62% from **5**).



Scheme 2. Reagents and conditions: (a) MEMCl, DIPEA, TBAI, CH_2Cl_2, rt, 17 h (**9**: 58%), NaHMDS, THF; then MeI, rt, 2 h (**10**: 45%); (b) MsCl, Et_3N, DMAP, CH_2Cl_2, rt, 24 h (93%); (c) $MeNH_2$, MeCN, reflux, 3 h (90%); (d) Boc_2O, MeCN, rt, 16 h (91%); (e) NaSH, MeOH/H_2O, reflux, 20 h; (f) 2-quinoxaloyl chloride, pyridine, $CHCl_3$, rt, 19 h (**12**: 59% from **9**), 2-quinoxaloyl chloride, pyridine, $CHCl_3$, rt, 22 h (**13**: 69% from **10**), 2-quinoxaloyl chloride, pyridine, $CHCl_3$, rt,16 h; then TFA, CH_2Cl_2, rt, 20 min (**14**: 55% from **11**).

constructed using the same procedure as Scheme 1 to afford new derivatives **12** and **13**. Methylamino derivative **14** was prepared via intermediacy **11**, which was obtained through a three-step sequence from the alcohol **4**.

We next gave our attention to the position of the quinoxaloylamide unit as a modifiable substructure of SRT1720. As shown in Scheme 3, *meta*- and *para*-nitrophenyl derivatives **17** and **18**, isomers of *ortho*-derivative **5**, were prepared through the essentially same reaction sequence as Scheme 1 via intermediates **15** and **16**, respectively. These compounds were successfully transformed into the desired *meta*- and *para*-amide-substituted SRT1720 analogues **19** and **20** (Scheme 3).



Scheme 3. Reagents and conditions: (a) Et_3N, 2-butanone, reflux, 24 h (**15**: 21%, **16**: 64%); (b) $NaBH_4$, EtOH, reflux, 20 h; (c) MsCl, Et_3N, DMAP, CH_2Cl_2, rt,18 h; (d) Boc-piperazine, Et_3N, MeCN, reflux, 18 h (**17**: 30% from **15**, **18**: 40% from **16**); (e) NaSH, MeOH/H_2O, reflux, 18 h; (f) 2-quinoxaloyl chloride, pyridine, $CHCl_3$, rt, 16 h; then TFA, CH_2Cl_2, rt, 2 h (**19**: 59% from **17**, **20**: 39% from **18**).

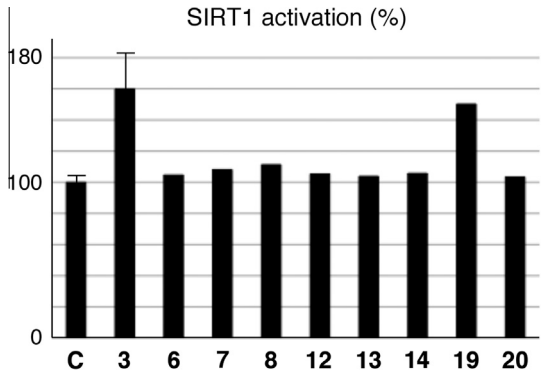
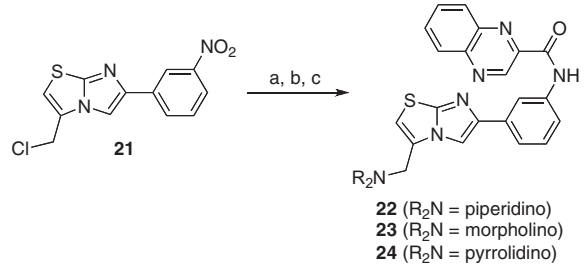
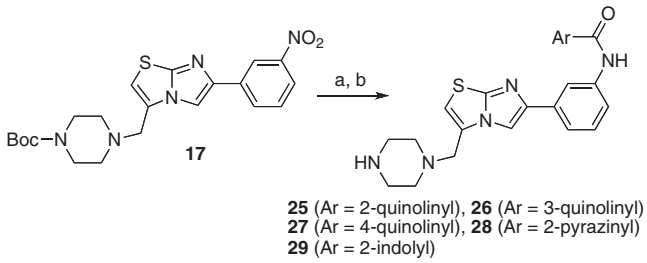


Figure 2. SIRT1 activation assay for SRT1720 and its derivatives, using SIRT1 fluorometric drug discovery kit (AK-555, BIOMOL Research Laboratories). **C** represents control experiment. Compound **3** is SRT1720 (positive control). For all experiments, compound concentrations were 10 μM. The SD bars for small value (below 2%) are omitted for clarity.



Scheme 4. Reagents and conditions: (a) R_2NH, Et_3N, MeCN, reflux, 16 h; (b) NaSH, MeOH/H_2O, reflux, 18 h; (c) 2-quinoxaloyl chloride, pyridine, $CHCl_3$, rt, 16 h (**22**: 67%, **23**: 53%, **24**: 14%, in three-steps, respectively).



Scheme 5. Reagents and conditions: (a) NaSH, MeOH/H_2O, reflux, 18 h; (b) $ArCO_2H$, EDC, DMAP, CH_2Cl_2, rt, 1 h; then TFA, CH_2Cl_2, rt, 1 h (**25**: 80%, **26**: 82%, **27**: 61%, **28**: 81%, **29**: 99%, in two-steps, respectively).

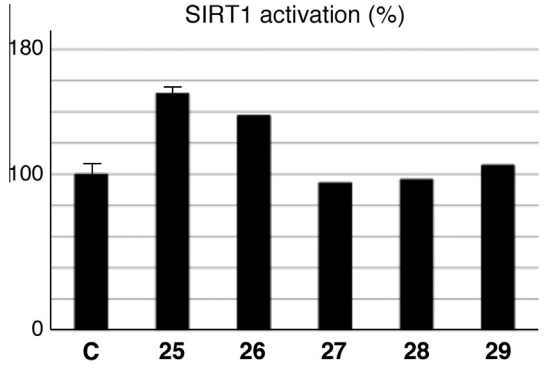


Figure 3. SIRT1 activation assay for the *m*-substituted derivatives **25**–**29**, using SIRT1 fluorometric drug discovery kit (AK-555, BIOMOL Research Laboratories). **C** represents control experiment. For all experiments, compound concentrations were 10 μM. The SD bars for small value (below 2%) are omitted for clarity.

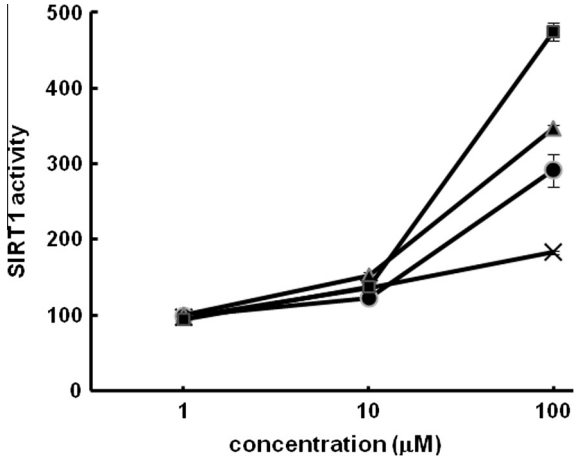


Figure 4. SIRT1 activation of compounds **3** (×), **19** (●), **25** (▲), and **26** (■) at 1, 10, and 100 μM.

With these new SRT1720 derivatives in hand, we screened their SIRT1 activation activities using a fluorometric in vitro assay method, and the results are summarized in Figure 2. At 10 μM concentration, the reference compound SRT1720 (**3**) exhibited ca. 160% activation. On the other hand, the other derivatives (**6**–**8** and **12**–**14**), all of which have *ortho*-amide-substituents, showed almost no activity at the same concentration. Gratifyingly, we could find that the *meta*-amide-substituted derivative **19** activated SIRT1 with comparable potency to SRT1720, whereas *para*-amide-substituted derivative **20** had no activity. These results prompted us to go ahead with further SAR studies focusing on the *meta*-amide-substituted structure.

The chloro-compound **21**, precursor of the compound **17**, was subjected to nucleophilic substitution reaction with three secondary amines (piperidine, morpholine, and pyrrolidine), and the products were converted to *meta*-quinoxaloyl-amide derivatives **22**–**24**, respectively, through *meta*-nitro group manipulations (Scheme 4). Modifications of aryl groups on the amide-substituents were also performed, starting from the piperazine derivative **17** with two steps, to furnish five derivatives **25**–**29** in satisfactory yields (Scheme 5). Thus, eight new SRT1720 derivatives possessing *meta*-amide-substituents were obtained.

Survey of the *meta*-amide-substituted derivatives **22**–**29** on SIRT1 activation was performed using the same in vitro assay method, and revealed that **22**–**24** did not exhibit any activities (data not shown), suggesting the piperazinyl moiety would be important for the activity. Concerning variation of the hetero-aromatic ring, 2-quinolinyl and 3-quinolinyl derivatives (**25** and **26**, respectively) exerted ca. 140–160% SIRT1 activation at 10 μM concentration, which was comparable to SRT1720 (**3**) and 2-quinoxalinyl derivative (**19**), and the other derivatives **27**–**29** were found to be inactive (Fig. 3).

Concentration dependency was examined for the active derivatives **19**, **25**, and **26**, as well as the reference compound SRT1720 (**3**). As summarized in Figure 4, all of the three new derivatives showed SIRT1 activation activity more potent than **3** especially at 100 μM. Thus, modified SRT1720 compounds with *meta*-amide-substituents were considered as promising candidates for a novel SIRT1 activator.

To explore the origin of the higher activity of the *meta* derivatives than that of SRT1720 (**3**), we studied the binding mode of **25** and SRT1720 with SIRT1 by using Molegro Virtual Docker 5 software.[9] Wu et al. have reported π/π interaction between an aromatic ring of activators and a coumarin ring of labeled substrate plays an important role for SIRT1 activation.[10] The two binding models in this Letter also indicate that the quinoxaline ring of SRT1720 and the quinoline ring of **25** can interact with the coumarin ring via π/π interaction (Fig. 5). In the simulated SRT1720/peptide substrate/SIRT1 complex, only one hydrogen bond is observed between the amide hydrogen atom of SRT1720 and the carbonyl oxygen atom of coumarin ring (Fig. 5A). On the other hand, two hydrogen bonds are observed in the simulated **25**/peptide substrate/SIRT1 complex, where there can be a hydrogen bond between the piperazine of **25** and the side chain of Gln 294 in SIRT1, in addition to the possibility of a hydrogen bond between the amide oxygen and the NH of Lys in the peptide substrate (Fig. 5B). These calculation results suggest that the two hydrogen bonds in the **25**/peptide substrate/SIRT1 complex may be attributed to its potent activity as compared with SRT1720.

In this Letter, we described syntheses and in vitro evaluation of new SIRT1 activator candidates based on SRT1720 possessing the imidazo[1,2-*b*]thiazole core, as a lead compound. Easily modifiable moieties of the SRT1720 structure, namely, piperazine moiety, quinoxaline ring on the amide group, and position of the amide function, were changed, and the assay results indicated that piperazine ring and hetero-bicyclic ring on the amide would be important for SIRT1 activation ability. Novel finding in this Letter is that *meta*-substitution pattern of the amide function increases the activity compared with *ortho*-substituted SRT1720, which can be rationalized by docking simulation of drug/peptide substrate/SIRT1 complex. In the docking study, compound **25** was docked into the catalytic site of SIRT1, however, it has recently been reported that SIRT1 activators may bind to an allosteric site of the enzyme.[11] Further studies including SIRT1 activation mechanism analysis of our activators will be continued in our laboratory.

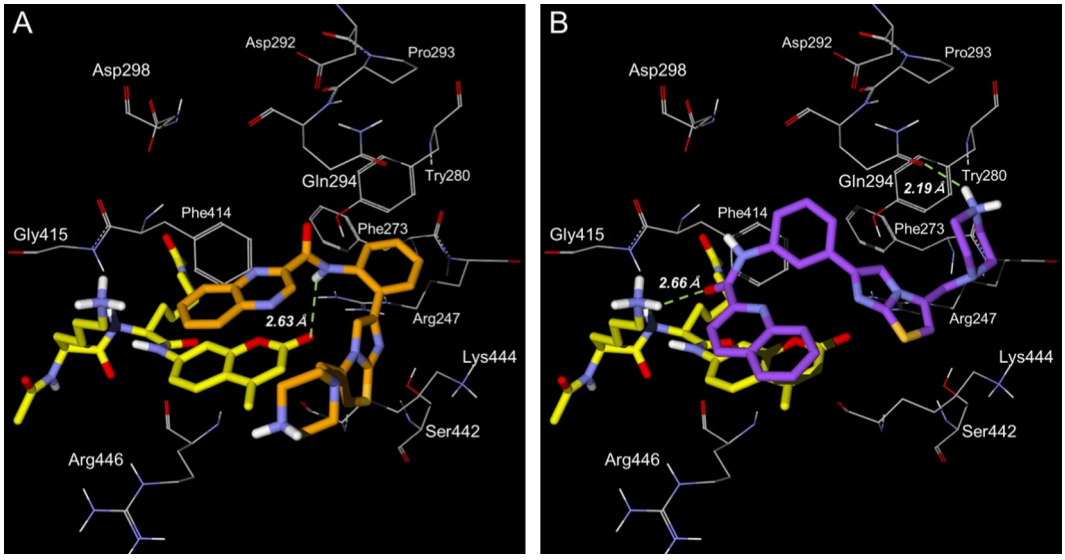


Figure 5. Modeling analysis of SRT1720 (A, orange) and derivative **25** (B, purple) bound to model complex of SIRT1 with fluorophore-labeled peptide substrate (yellow).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.06.070.

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