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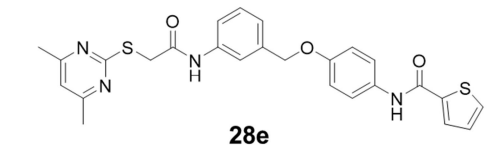

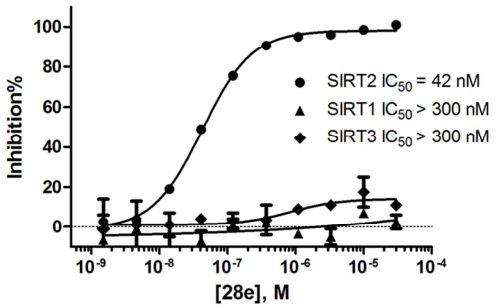

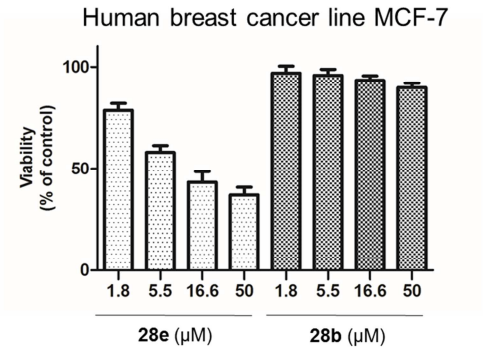

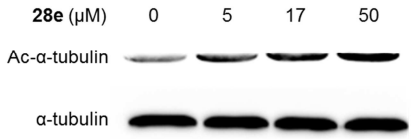

Discovery of 2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-phenylacetamide derivatives as new potent and selective human Sirtuin 2 inhibitors

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Abstract

Human sirtuin 2 (SIRT2) plays pivotal roles in multiple biological processes such as cell cycle regulation, autophagy, immune and inflammatory responses. Dysregulation of SIRT2 was considered as a main aspect contributing to several human diseases, including cancer. Development of new potent and selective SIRT2 inhibitors is currently desirable, which may provide a new strategy for treatment of related diseases. Herein, a structure-based optimization approach led to new 2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-phenylacetamide derivatives as SIRT2 inhibitors. SAR analyses with new synthesized derivatives revealed a number of new potent SIRT2 inhibitors, among which **28e** is the most potent inhibitor with an IC_{50} value of 42 nM. The selectivity analyses found that **28e** has a very good selectivity to SIRT2 over SIRT1 and SIRT3. In cellular assays, **28e** showed a potent ability to inhibit human breast cancer cell line MCF-7 and increase the acetylation of α-tubulin in a dose-dependent manner. This study will aid further efforts to develop highly potent and selective SIRT2 inhibitors for the treatment of cancer and other related diseases.

Keywords: Sirtuins; SIRT2; Epigenetics; Cancer; Structure-based optimization

1. Introduction

Sirtuins (SIRTs) were initially recognized as NAD^+-dependent protein deacetylases that remove acetyl groups from ε-*N*-acetyl-lysine amino groups on histones and non-histone substrates, e.g. *α*-tubulin[1,2]. Recently, sirtuins were found to catalyze further post-translational modifications such as desuccinylation[3], demalonylation[3], demyristoylation[4], and ADP-ribosylation[5,6]. By modifying various substrates, sirtuins are involved in multiple crucial physiological processes, including the regulation of metabolism, transcription, and DNA damage repair[1,2,7-10]. In human the sirtuin family comprises seven proteins (SIRT1-SIRT7), which vary in their catalytic activity and subcellular localization. The human sirtuin 2 (SIRT2), located in both cytoplasm and nucleus, mainly catalyzes deacetylation or demyristoylation for a variety of protein substrates, including histones H3[11] and H4[12], and non-histone proteins *α*-tubulin[13], p53[14], p65[15], Foxo1[16], and Foxo3a[17]. SIRT2 thus has been shown to play important roles in cell cycle regulation, autophagy, peripheral myelination, immune and inflammatory response[1,7,8].

Overexpression or dysregulation of SIRT2 was considered as a main aspect contributing to several human diseases, including cancer[1,8]. For example, Chen *et al* recently found that SIRT2 is overexpressed in hepatocellular carcinoma, and mediates epithelial to mesenchymal transition (EMT) by regulating the deacetylation of protein kinase B and subsequently influencing the glycogen synthase kinase-3β/β-catenin signaling pathway[18]. Many other studies revealed that SIRT2 is up-regulated in several human cancers such as breast cancer and pancreatic

adenocarcinomas, and SIRT2 down-regulation or inhibition leads to cancer cell apoptosis or growth suppression[19,20]. Although SIRT2 was also observed to have cancer-suppressing activity in some types of cancer[21], the development of SIRT2 inhibitors, especially potent and selective inhibitors, would be an impetus to probe associated molecular mechanism and to develop therapeutic drugs for the treatment of selected cancer types.

To date, various structurally diverse types of SIRT2 inhibitors have been reported (**1**-**11**, Figure 1)[22-28], most of which showed only moderate potency or non-specific inhibition to SIRT2. Notably, Jung and coworkers recently discovered aminothiazoles as selective SIRT2 inhibitors termed SirReals (**9**, Figure 1), which can induce/bind to a new selective binding pocket (surrounded by almost all the hydrophobic residues such as Phe96, Phe190, Ile169, Leu134, Ile232, Phe119, Leu138, Tyr139, Pro140, and Phe143) as revealed by crystallographic analyses[27,29,30].

With the aim of identifying new selective SIRT2 inhibitors, we initially carried out structure-based design by LEADOPT, an automatic lead optimization tool developed recently by us, which enables generation of new derivatives for the given lead compound and its core scaffold according to the target information (details can be found in our previous work[31]). The Sirt2:SirReal2 (**9**, Figure 1) complex structure (PDB ID: 4RMG)[29] was selected as the template, and the 2-((4,6-dimethylpyrimidin-2-yl)thio)acetamide motif (shown in red, Figure 2) was defined as the core scaffold. The LEADOPT computations led to the generation of 1341 molecules, among which 422 molecules have a ligand efficiency (LE) score

larger than 0.38 (an empirical LE score) and an ADMET score larger than 9 (an ADMET score of SirReal2). From them, we selected compounds **I** and **II** to synthesize (Figure 2) because both have never been reported and can be readily synthesized (Scheme 1); the moieties of **I** and **II** (3-phenoxyphenyl and 3-((thiophen-2-ylmethyl)amino)phenyl, respectively) introduced by LEADOPT appear positioned to form hydrophobic interactions with Phe96, Phe190, Ile169, Leu134, Ile232, and Phe119. The inhibition assays showed that **I** and **II** displayed 92±3% and 93±1% inhibition against SIRT2 at 50 μM, and 49±5% and 60±2% inhibition at 5 μM (Table 1), respectively. These preliminary results indicated that **I** and **II** could be as potential hit compounds for further structural optimization to obtain potent and selective SIRT2 inhibitors. We herein describe structure-activity relationship (SAR) of 2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-phenylacetamide (the core chemical scaffold of compounds **I** and **II**) derivatives with SIRT2, and anti-cancer activity for the most potent compound.

<**Figure 1 here**>

<**Figure 2 here**>

2. Results and Discussion

2.1 Synthesis of 2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-phenylacetamide derivatives.

We synthesized a series of 2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-phenyl acetamide derivatives to study the SAR with SIRT2 by the synthetic routes outlined in Schemes 1-3. A general scheme for the synthesis of compounds **14a-j** and **15a-b** is shown in

Scheme 1. Reactions of commercially available 3-/4-substituted anilines (**12a-b**, **12d**, and **12g-h**) or synthesized 3-/4-substituted anilines (**12c**, **12e-f**and **12i-j**, see Scheme S1) with 2-bromoacetic acid in the presence of 1-hydroxybenzotriazole (HOBT), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDCI), and *N*,*N*-diisopropylethylamine (DIPEA), produced the condensation products **13a-j**. The resulting key intermediates **13a-j** were subsequently reacted with 4,6-dimethylpyrimidine-2-thiolin the presence of *t*-BuoK at room temperature to give the desired compounds **14a-j** in 56%-88% yields. Further oxidization of compound **14b** by 1.0 equiv or 3.0 equiv *m*-CPBA led to the white solid compounds **15a-b**, respectively.

The synthesis of compounds **19a-f** and **24a-b**, which contain a CH_2-NH or NH-CH_2 linker substituted at the benzene ring of 2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-phenyl acetamide, are depicted in Scheme 2. The compounds **19a-f** containing a CH_2-NH linker, were obtained by starting from the condensation reaction between *p*/*m*-phenylenediamine with 2-bromoacetic acid, selectively. Then, the nucleophilic substitution and reductive amination reaction were performed in sequence to produce the final products **19a-f** in high yields. For the NH-CH_2 linker compounds **21a-b**, a two-step reductive amination reaction between 3/4-nitrobenzaldehyde with aniline resulted in the intermediates **21a-b**. Then the target compounds **24a-b** were synthesized from **21a-b** using a method similar to that for **14a-j** in moderate yields.

Scheme 3 presents the synthetic routes for compounds **28a-k**, **32** and **35**, which

contain an O-CH_2 or CH_2-O linker. For the synthesis of O-CH_2 linker compounds **28a-k**, the target compound **28e** was described as an example. First, commercially available1-(bromomethyl)-3-nitrobenzene (**25**) was reacted with *N*-(4-hydroxyphenyl)acetamide by a base-promoted nucleophilic substitution followed by a deacetylation reaction in the presence of thionyl chloride and methanol. Next, the free amino group was reacted with thiophene-2-carbonyl chloride in a standard condensation of chloride and amine to yield the key intermediates **26e,** which was reduced to give the compound **27e** in a high yield. Finally, **28e** was obtained using a two-step procedure involving condensation reaction and final nucleophilic substitutionas described above. For the CH_2-O linker contained compounds **32** and **35**, intermediates 3-(benzyloxy)aniline (**31**) and 4-(benzyloxy)aniline (**34**) were used to react with 2-bromoacetic acid and 4,6-dimethylpyrimidine-2-thiol by general procedure 1 and general procedure 2 (see experimental section) in turn, respectively. Note, the key intermediates **31** and **34** were synthesized through different synthesis methods, which were described in the experimental section.

2.2 SAR studies with SIRT2.

In order to explore SAR of 2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-phenylacetamide derivatives with SIRT2, we firstly synthesized compounds **14a-j** (Table 1) containing oxydibenzene, diphenylamine, diphenylmethane, benzophenone and diphenylmethanol at 3- or

4-position of the phenyl of 2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-phenylacetamide scaffold (Table 1). Compared with the hit compound **I** (**14a**), which has an oxydibenzene motif at the 3-position, compound **14b**, containing an oxydibenzene motif at 4-position, showed slightly lower potency to inhibit SIRT2 (78±3% and 42±4% inhibition at 50μM and 5 μM, respectively). A further comparison of the compound pairs [**14c** vs **14d**], [**14e** vs **14f**], [**14g** vs **14h**], and [**14i** vs **14j**], which contain diphenylamine, diphenylmethane, benzophenone and diphenylmethanol at 3- or 4-position, respectively, revealed that 3-substituted derivatives are likely to have more potent inhibition against SIRT2 than 4-substituted derivatives (Table 1). Notably, **14e**, containing a diphenylmethane motif at 3-position, displayed promising potency with 96±3% and 73±4 % SIRT2 inhibition at 50μM and 5 μM, respectively, which is more potent than the known SIRT2 inhibitor Suramin (Table 1). Furthermore, we synthesized compounds **15a** and **15b**, which are the oxidized form on thio group of **14b**. Comparing with **14b**, the activities of compounds **15a** and **15b** against SIRT2 significantly decreased (Table 1), indicating that the oxidized form of the 2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-phenylacetamide may destruct the intermolecular hydrogen bond between dimethylpyrimidine and amide, which is an very important feature of the inhibition mode as observed in previous crystallographic analyses[29,30].

<**Table 1 here**>

According to the hit compound **II** (**19a**), we synthesized the target compounds **19b-f** (CH_2-NH linker), **24a-b** (NH-CH_2 linker), **32** (CH_2-O linker), **35**(CH_2-O linker),

and **28a-b** (O-CH_2 linker) (Table 1), which contain ((thiophen-2-ylmethyl)amino)phenyl, (benzyloxy)phenyl, ((phenylamino)methyl) phenyl, (benzylamino)phenyl, ((pyridin-4-ylmethyl)amino)phenyl, ((furan-2-yl methyl)amino)phenyl and (phenoxymethyl)phenyl at 3- or 4-position. We observed that **19b**, bearing a ((thiophen-2-ylmethyl)amino)phenyl motif at 4-position, show lower SIRT2 inhibition than the hit compound **II** (**19a**), which has a ((thiophen-2-ylmethyl)amino)phenyl motif at 3-position. Consistent with the SAR described above, compounds **19c**, **24a**, **32**, and **28a**, which have (benzyloxy)phenyl, ((phenylamino)methyl)phenyl, (benzylamino)phenyl, and (phenoxymethyl)phenyl at 3-position, respectively, manifested more potent inhibition against SIRT2 than compounds **19d**, **24b**, **36**, and **28b** with corresponding substituents at 4-position. These results indicated that substituents at 3-position of the phenyl of 2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-phenylacetamide may be beneficial to fit with the induced selective SIRT2 binding pocket. Notiably, among these tested compounds, **28a** with a (phenoxymethyl)phenyl moiety at 3-position showed the very potent inhibition towards SIRT2 (71±3% and 96±3% inhibition at 5μM and 50μM, respectively). Molecular docking analyses indicated that **28a** appears positioned to make good hydrophobic interactions with Phe119, Ile232, Phe131, Leu134, Leu138, and Phe96 (Figure 3a); compared with compound **I**, the (phenoxymethyl)phenyl moiety of **28a** likely binds adjacent to the hydrophilic region (such as Val233 and His187, Figure 3a), suggesting that modification of the A moiety or introduction of substituents at 3- or 4-position of the A moiety of **28a** (Table 2) would probably

improve SIRT2 inhibitory activity.

<**Figure 3 here**>

<**Table 2 here**>

We further synthesized compounds **28c-k**, which contain various substituents on or variants of the phenyl moiety (the A moiety, Table 2) of **28a**, using the routes given in Scheme 3. Inhibition assays revealed that substituents at the A moiety of **28a** showed comparable or improved SIRT2 inhibitory activities to **28a** (Table 2). Compound **28e**, which contains a thiophene-2-carboxamide moiety as the A moiety of **28a**, displayed very potent inhibition to SIRT2 (98 % inhibition at 5μM). We also observed that compounds **28h**, **28i**, **28j**, and **28k**, which have 1-naphthalene, 2-naphthalene, 8-quinoline, or 5-1*H*-indazole as the A moiety, showed moderate SIRT2 inhibitory activity but better than Surinam. Through molecular docking anlayses, we observed that, comparing with **28a**, the thiophene-2-carboxamide moiety of **28e** is likely to have additional hydrogen-bonding interactions with Val233 and π-π stacking interactions with His187, which may explain why **28e** has more potent inhibition activity than **28a**. Together, these SAR studies led to a series of new 2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-phenylacetamide derivatives as SIRT2 inhibitors, and especially the most potent compound **28e**.

2.3 The selectivity of compound 28e.

For compound **28e**, we further determined the IC_{50} value against SIRT2. As shown in Figure 4a, **28e** inhibit SIRT2 via a dose-dependent manner with an IC_{50} value of 42nM, which is more potent than Suramin (with an IC_{50} value of 14 μM,

Figure 4b). We further tested compound **28e** against SIRT1 and SIRT3, which are close analogues to SIRT2, with the aim of testing the selectivity of **28e**. We observed that **28e** did not show obvious inhibition to SIRT1 and SIRT3 even at the highest concentration of 300 μM (Figure 4c and 4e); for comparison, the SIRT1 inhibitor EX-527 and SIRT3 inhibitor SRT1720 displayed potent inhibition with IC_{50} values of 0.13 μM and 0.85 μM, respectively, under the same assay conditions (Figure 4d and 4f). These results indicated that **28e** has good selectivity to SIRT2. It is possible that **28e** as well as other derivatives (e.g. **28a**) probably inhibit SIRT2 via binding to the selective binding pocket[29,30].

<**Figure 4 here**>

2.4 Cellular Activities of 28e.

We tested whether compound **28e** inhibit human breast cancer line MCF-7, which was reported to be associated with the SIRT2 activity[26]. MCF-7 cells were treated with **28e** in four different concentrations (1.8 μM, 5.5 μM, 16.6 μM, and 50 μM) for 120 hours. We observed that compound **28e** displayed a dose-dependent inhibition to MCF-7 with more than 50% inhibition at 50 μM (Figure 5a). For comparison, we tested compound **28b** with a (phenoxymethyl)phenyl moiety at 4-position (Table 2), which shows much lower inhibitory activity against SIRT2 than **28e**; **28b** did not show obvious inhibition against MCF-7 at 50 μM (Figure 5a). Meanwhile, we tested the inhibitory acitvities of compounds **28e** and **28b** against human normal liver cell line HL-7702, which was used for ruling out the activities due cytotoxicity, We observed that compounds **28e** and **28b** had no activity against HL-7702 at 100μM

(with 3% and 6% inhibition @100μM, respectivly), suggesting that **28e** and **28b** are not cytotoxic compounds. We then examined the effect of **28e** on the acetylation of *α*-tubulin, one of the key protein substrate of SIRT2. The results from western blotting analysis showed that the acetylation of *α*-tubulinin MCF-7 cells was increased after **28e** treatment for 6h (Figure 5b). These results indicated that **28e** may inhibit MCF-7 by suppressing the SIRT2 activity and related signaling pathways.

<**Figure 5 here**>

3. Conclusion.

A series of 2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-phenylacetamide derivatives were synthesized according to the hit compounds **I** and **II** obtained by LEADOPT. The SAR analyses on these synthesized derivatives led to the identification of a number of new potent SIRT2 inhibitors, e.g. **28e** (with an IC_{50} value of 42 nM). The selectivity analyses revealed **28e** has a good selectivity to SIRT2 over SIRT1 and SIRT3. **28e** displayed inhibition to MCF-7 in a dose-dependent manner, which showed a potent ability to increase the acetylation of *α*-tubulin as observed in western blotting analyses. This study will aid further efforts to discover highly potent and selective SIRT2 inhibitors and to develop SIRT2 inhibitors as a newstrategy for the treatment of cancer as well as other associated diseases.

4. Experimental

Chemistry Methods

All chemicals were obtained from commercial purchase and used as supplied without further purification. Reactions were monitored by thin layer chromatography (TLC) on Merck silica gel 60 F-254 thin layer plates. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer at 400 MHz and 100 MHz, respectively. Chemical shifts are given in ppm (d) relative to tetramethylsilane as an internal standard or the NMR solvent. Low-resolution and high-resolution mass spectral (MS) data were acquired on an Agilent 1100 series LC-MS instrument with UV detection at 254 nm in electrospray ionization (ESI) mode. Melting points were measured on an electrothermal melting point apparatus without correction. All the target compounds were purified to >95% purity, as determined by high-performance liquid chromatography (HPLC). HPLC analysis was performed on a Waters 2695 HPLC system equipped with a Kromasil C18 column (4.6 mm × 250 mm, 5 um).

General procedure 1: EDCI-mediated amide formation

A mixture of 2-bromoacetic acid(1.0 equiv), 1-hydroxybenzotriazole (HOBT,1.0 equiv), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDCI, 1.0 equiv) and *N*,*N*-diisopropylethylamine (DIPEA, 1.5 equiv) in dichloromethane (DCM) was stirred at ambient temperature for 0.5 h. To the solution of the mixture, different amines (1.0 equiv) were added, and the reaction mixture was continued to stir 10-12 h at room temperature[32,33]. Upon completion of the reaction as determined by TLC, The mixture was concentrated and partitioned between water and ethyl acetate. The organic layer was dried over magnesium sulfate anhydrous, filtered and concentrated in *vacuo*. The products were purified bycolumn chromatography with appropriate eluents.

General procedure 2: *t*-BuOK-catalyzed nucleophilic substitution

A solution of the 2-bromoacetamides (1.0 equiv), obtained via general procedure1, 4,6-dimethylpyrimidine-2-thiol (1.2 equiv) and *t*-BuOK (2.0 equiv) in DMF (10 mL/mmol) was stirred at ambient temperature for 5-12h. When TLC indicated that the reaction was finished, the reaction mixture was poured into a large amount of water (20ml/1ml DMF) and the crude mixture was extracted with ethyl acetate (3×). Then, the combined organic layers were washed with brine, dried, and concentrated. The residue was purified by column chromatography to give the desired target compounds or key intermediates.

2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-(3-(phenylamino)phenyl)acetamide (14c)

A mixture of 3-nitroaniline (1.2 equiv), phenylboronic acid (1.0 equiv), DBU (2.0 equiv), $NiCl_2.6H_2O$ (0.2 equiv), and 2,2'-$(C_5H_4N)_2$ (equiv) in MeCN (10ml/mmol) was stirred for 28h at room temperature, Upon completion of the reaction as determined by TLC, The mixture was concentrated and partitioned between water and ethyl acetate. The organic layer was dried over magnesium sulfate anhydrous, filtered and concentrated in *vacuo*. The solid was purified by column chromatography to give the key intermediate **12cb**. Though the Fe-mediated reduction reaction, the N^1-phenylbenzene-1,3-diamine (**12c**) was synthesized (Scheme S1). Using **12c**, the title compound **14c** was obtained by general procedure 1 and general procedure 2 in turn, 49% for two steps, 97.1% HPLC purity. mp: > 300°C; ^{1}H NMR (400 MHz, DMSO): δ 10.12 (s, 1H), 8.18 (s, 1H), 7.46 (s, 1H), 7.24 (t, *J*=8.0 Hz, 2H), 7.14 (t, *J*=8.0 Hz, 1H), 7.08 (d, *J*=7.6 Hz, 2H), 7.01 (d, *J*=8.8 Hz, 1H), 6.98 (s, 1H), 6.83 (t, *J*=7.2 Hz, 1H), 6.74 (dd, *J*=1.6 Hz, *J*=5.6 Hz, 1H), 4.02 (s, 2H),2.35 (s, 6H) ppm. ^{13}C

NMR (100 MHz, DMSO): δ 169.8, 167.4, 166.9, 140.3, 140.0, 139.5, 129.0, 128.8, 128.6, 125.5, 124.1, 119.7, 117.4, 116.5, 35.9, 23.8 ppm. HRMS: m/z calcd for $C_{20}H_{21}N_4OS$ $[M + H]^+$ 365.1431, found 365.1441; $C_{20}H_{20}N_4NaOS$ $[M + Na]^+$ 387.1250, found 387.1251.

2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-(4-(phenylamino)phenyl)acetamide(14d)

Using the commercially available N^1-phenylbenzene-1,4-diamine (**12d**), the title compound **14d** was obtained by general procedure 1 and general procedure 2 in turn, 48% for two steps, 96.8% HPLC purity. mp: > 300°C; ^{1}H NMR (400 MHz, DMSO): δ 10.08 (s, 1H),8.05 (s, 1H), 7.46 (d, *J*=8.8 Hz, 2H), 7.20 (t, *J*=8.0 Hz, 2H), 7.05-6.98 (m, 5H), 6.77 (t, *J*=7.2 Hz, 1H), 4.02 (s, 2H), 2.35 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO): δ 169.8, 167.4, 166.3, 144.5, 139.4, 132.3, 129.6, 121.0, 119.5, 118.3, 116.5, 116.3, 35.9, 23.8 ppm. HRMS: m/z calcd for $C_{20}H_{21}N_4OS$ $[M + H]^+$ 365.1431, found 365.1442; $C_{20}H_{20}N_4NaOS$ $[M + Na]^+$ 387.1250, found 387.1251.

***N*-(4-benzylphenyl)-2-((4,6-dimethylpyrimidin-2-yl)thio)acetamide (14f)** To a mixture of $LiAlH_4$ (10.0 equiv) in Et_2O (5ml/mmol) was added the solution of $AlCl_3$ (10.0 equiv) in Et_2O (10ml/mmol) slowly at 0°C , and the reaction mixture was stirred for 5 min. Then the solution of (4-aminophenyl)(phenyl)methanone (**12h**, 1.0 equiv) in Et_2O (15ml/mmol) was added, and the mixture was stirred at room temperature for 3 h. After completion (monitored by TLC), the mixture was diluted using 6M HCl and neutralized by saturated sodium bicarbonate solution. Next, the solution was partitioned between water and ethyl acetate (3×). The organic layer was dried over magnesium sulfate anhydrous, filtered and concentrated in *vacuo*. The solid was

purified by column chromatography to give the key intermediate **12f** in 68% yield (Scheme S1). Using **12f**, the title compound **14f** was obtained by general procedure 1 and general procedure 2 in turn, 53% for two steps, ^{1}H NMR (400 MHz, DMSO): δ10.17(s, 1H),7.49 (d, *J*=8.4 Hz, 2H), 7.28 (t, *J*=7.6 Hz, 2H), 7.22-7.15 (m, 5H), 6.97 (s, 1H), 4.02 (s, 2H), 3.89 (s, 2H), 2.34 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO): δ 169.8, 167.4, 166.8, 141.9, 137.5, 136.7, 129.4, 129.1, 128.8, 125.4, 119.8, 116.5, 40.0, 35.9, 23.8 ppm. HRMS: m/z calcd for $C_{20}H_{21}N_4OS$ $[M + H]^+$ 364.1516, found 364.1513; $C_{21}H_{21}N_3NaOS$ $[M + Na]^+$ 386.1298, found 386.1295.

***N*-(3-benzoylphenyl)-2-((4,6-dimethylpyrimidin-2-yl)thio)acetamide (14g)** Using the commercially available(3-aminophenyl)(phenyl)methanone (**12g**), the title compound **14g** was obtained by general procedure 1 and general procedure 2 in turn, 45% for two steps, 96.9% HPLC purity. ^{1}H NMR (400 MHz, $CDCl_3$): δ9.84(s, 1H), 8.02 (t, *J*=8.0 Hz, 1H), 7.80 (d, *J*=8.4 Hz, 2H), 7.66 (s, 1H), 7.62 (t, *J*=7.2 Hz, 1H), 7.52-7.43 (m, 4H), 6.85 (s, 1H), 3.91 (s, 2H), 2.50 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO): δ 196.0, 169.7, 167.4, 139.6, 138.0, 137.5, 133.1, 130.0, 129.6, 129.0, 124.9, 123.5, 120.6, 116.5, 35.9, 23.8 ppm. LCMS m/z: 378.1 $[M+H]^+$.

2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-(4-(hydroxy(phenyl)methyl)phenyl)aceta mide (14j) Firstly, according to the synthesis method for compound **12f**, using 5.0 equiv $LiAlH_4$ and $AlCl_3$ the intermediate **12j** was synthesized in 73% yield (Scheme S1); Then, using **12j**, the target compound **14j** was got by general procedure 1 and general procedure 2 in turn, 55% for two steps, 97.6% HPLC purity. ^{1}H NMR (400 MHz, DMSO): δ 10.20(s, 1H),7.57 (s, 1H), 7.47 (d, *J*=8.0 Hz, 1H), 7.36 (d, *J*=7.2 Hz,

2H), 7.30 (t, *J*=8.0 Hz, 2H), 7.25-7.19 (m, 2H), 7.07(d, *J*=7.6 Hz, 1H), 6.97 (s, 1H), 5.89 (d, *J*=4.0 Hz, 1H), 5.65 (d, *J*=4.0 Hz, 1H), 4.01 (s, 2H), 2.32 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO): δ 169.8, 167.4, 166.9, 146.9, 146.0, 140.2, 128.9, 128.3, 127.2, 125.3, 121.8, 119.9, 118.1, 117.6, 116.5, 74.8, 36.0, 23.8 ppm. HRMS: m/z calcd for $C_{21}H_{22}N_3O_2S$ $[M + H]^+$ 380.1427, found 380.1435; $C_{21}H_{21}N_3NaO_2S$ $[M + Na]^+$ 402.1247, found 402.1257.

General procedure 3: *m*-CPBA-involved oxidation reaction

To a solution of 2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-(4-phenoxyphenyl)acetamide (**14b**, 1.0 equiv) in DCM (20ml/1mmol) at 0°C was added 3-chlorobenzenecarboperoxoic acid (*m*-CPBA, 1.05 equiv), then the mixture was allowed to warm to room temperature and stirred for 45 min. The resulting solution was concentrated under reduced pressure to dryness and the residue was purified by silica gel column chromatography to give the white finial compounds **15a** in 62% yield, 97.3% HPLC purity. mp: > 300°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.32(s, 1H),7.47 (d, *J*=8.8 Hz, 2H), 7.33 (t, *J*=7.6 Hz, 2H), 7.10 (t, *J*=8.8 Hz, 2H), 7.00-6.93 (m, 5H), 4.16 (d, *J*=14.4 Hz, 1H), 3.93 (d, *J*=14.4 Hz, 1H), 2.55 (s, 6H) ppm.^{13}C NMR (100 MHz, DMSO): δ 171.5, 168.9, 162.9, 157.7, 152.7, 134.9, 130.4, 123.5, 121.9, 121.4, 119.9, 118.4, 60.5, 23.9 ppm. HRMS: m/z calcd for $C_{20}H_{19}N_3NaO_3S$ $[M + Na]^+$ 404.1039, found 404.1030.

Similarly, to a solution of compounds **14b** (1.0 equiv) in DCM (20ml/1mmol) at 0°C was added 3-chlorobenzenecarboperoxoic acid (*m*-CPBA, 3.0 equiv), then the mixture was allowed to warm to room temperature and stirred for 3h. Upon

completion of the reaction as determined by TLC, the resulting solution was concentrated under reduced pressure to dryness and the residue was purified by silica gel column chromatography to give the white finial compounds **15b** in 71% yield, 97.6% HPLC purity.mp: > 300°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.76 (s, 1H),7.48 (d, *J*=8.8 Hz, 2H), 7.35 (t, *J*=8.0 Hz, 2H), 7.28 (s, 1H), 7.12 (t, *J*=8.8 Hz, 1H), 7.01-6.98 (m, 4H), 4.56 (s, 2H), 2.63 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO): δ 169.3, 164.5, 159.8, 157.6, 152.9, 134.6, 130.5, 123.7, 123.6, 121.4, 119.9, 118.5, 57.9, 23.8 ppm. HRMS: m/z calcd for $C_{20}H_{20}N_3O_4S$ $[M + H]^+$ 398.1169, found 398.1159; $C_{20}H_{19}N_3NaO_4S$ $[M + Na]^+$ 420.0988, found 420.0980.

General procedure 4: Hantzsch-involved reductive amination

Catalytic amount of molecular sieve and trifluoroacetic acid were added to the solution of substituted anilins (1.0 equiv), different aromaticaldehydes (1.2 equiv), and diethyl 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (Hantzsch, 1.2 equiv) in CH_2Cl_2 at room temperature, and the reaction was warmed to 45°C and reacted for 12 h. After completion (monitored by TLC), the reaction was filtered, and the crude residue was obtained by concentrating the filtrate in *vacuo*. Finally, the crude residue was purified by column chromatography to give the intermediate or target compounds in high yield.

General procedure 5: Fe-mediatedreduction reaction

The nitro compounds (1.0 equiv) were taken in EtOH (6 mL/mmol), and iron powder (5.0 equiv) was added at 50°C-55 °C followed by NH_4Cl solution (0.5 equiv in 3 mL/mmol water). The reaction mixture was refluxed for about 1 h[33,34]. When TLC

indicated that the reaction was finished, most of the iron powder was filtered while hot and the alcohol was removed under reduced pressure. The residue was basified with $NaHCO_3$ solution (pH 7-8) and extracted several times with ethyl acetate, the combined organic extracts were dried (Na_2SO_4), and concentrated under reduced pressure to yield the reduction product. These materials were taken up for the next step without any purification.

2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-(4-((thiophen-2-ylmethyl)amino)phenyl)acetamide (19b) The title compound was obtained by the general procedures as above, 19% for four steps, 96.7% HPLC purity. ^{1}H NMR (400 MHz, DMSO): δ 9.80(s, 1H),7.32 (d, *J*=3.2 Hz, 1H), 7.24 (d, *J*=5.6 Hz, 2H), 7.01 (s, 1H), 6.93 (t, *J*=3.2 Hz, 2H), 6.57 (d, *J*=5.6 Hz, 2H), 6.07 (br s, 1H,), 4.39 (s, 2H), 3.95 (s, 2H), 2.32 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO): δ 170.8, 169.9, 167.4, 165.9, 145.1, 144.8, 129.2, 127.2, 125.2, 124.8, 121.3, 116.5, 113.0, 55.4, 35.8, 23.8 ppm. LCMS m/z: 385.1 $[M+H]^+$.

2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-(4-((pyridin-4-ylmethyl)amino)phenyl)acetamide (19f) The title compound was obtained by the general procedures as above, 21% for three steps, 96.6% HPLC purity. ^{1}H NMR (400 MHz, DMSO): δ 9.83(s, 1H), 8.48 (d, *J*=5.6 Hz, 2H), 7.33 (d, *J*=6.0 Hz, 2H), 7.24 (d, *J*=8.8 Hz, 2H), 6.97 (s, 1H), 6.50 (d, *J*=8.8 Hz, 2H), 6.29 (t, *J*=6.0 Hz, 1H), 4.29 (d, *J*=6.4 Hz, 2H), 3.96 (s, 2H), 2.32 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO): δ169.9, 167.4, 166.0, 150.2, 149.9, 145.1, 129.0, 122.8, 121.4, 116.5, 112.7, 46.1, 35.7, 23.8 ppm. LCMS m/z: 380.2 $[M+H]^+$.

2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-(3-((phenylamino)methyl)phenyl)acetamide (24a) The title compound was obtained by the general procedures as above, 30% for four steps, 96.9% HPLC purity. mp: > 300°C; ^{1}H NMR (400 MHz, DMSO): δ 10.21(s, 1H),7.55 (s, 1H), 7.48 (d, *J*=8.4 Hz, 1H), 7.25 (t, *J*=8.0 Hz, 1H), 7.06-7.01 (m, 3H), 6.96 (s, 1H), 6.55-6.49 (m, 3H), 6.24 (d, *J*=6.0 Hz, 1H), 4.22 (d, *J*=6.0 Hz, 2H), 4.02 (s, 2H), 2.32 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO): δ 169.8, 167.4, 166.9, 149.1, 141.6, 139.6, 129.3, 129.1, 122.6, 118.2, 118.0, 116.5, 116.2, 112.7, 47.0, 35.9, 23.8 ppm. LCMS m/z: 379.2 $[M+H]^{+}$.

2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-(3-(phenoxymethyl)phenyl)acetamide (28a) 1-(Bromomethyl)-3-nitrobenzene (1.0 equiv) and phenol (1.05 equiv) were dissolved in DMF (2ml/mmol) and reacted at room temperature for 12h, in the presence of K_2CO_3 (3.0 equiv). After water (50ml/mmol) was added, the mixture was partitioned between water and ethyl acetate (3×). The organic layer was dried over magnesium sulfate anhydrous and concentrated in *vacuo*. The residue was purified by column chromatography (eluent gradient PE/EA= 8/1) to give the intermediate **26a** in 82% yield. Using **26a**, the title compound was obtained by the general procedures as above, 27% for four steps, 97.7% HPLC purity. mp: > 300°C; ^{1}H NMR (400 MHz, DMSO): δ 10.29(s, 1H), 7.69 (s, 1H), 7.54 (d, *J*=8.4 Hz, 1H), 7.35-7.28 (m, 3H), 7.14 (d, *J*=7.6 Hz, 1H), 7.01-6.93 (m, 4H), 5.08 (s, 2H), 4.04 (s, 2H), 2.33 (s, 6H)ppm. ^{13}C NMR (100 MHz, DMSO): δ 169.8, 167.4, 167.1, 158.7, 139.7, 138.3, 130.0, 129.3, 122.9, 121.2, 119.0, 118.5, 116.5, 115.2, 69.5, 35.9, 23.8 ppm. HRMS: m/z calcd for $C_{21}H_{22}N_3O_2S$ $[M + H]^{+}$ 380.1427, found 380.1441; $C_{21}H_{21}N_3NaO_2S$ $[M + Na]^{+}$

402.1274, found 402.1259.

2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-(4-(phenoxymethyl)phenyl)acetamide (28b) The target compound was obtained using a method similar to that for **28a**, 26% for four steps, 96.3% HPLC purity. ^{1}H NMR (400 MHz, DMSO): δ 10.30(s, 1H), 7.61 (d, *J*=8.4 Hz, 2H), 7.40 (d, *J*=8.4 Hz, 2H), 7.30 (t, *J*=7.2 Hz, 2H), 7.01-6.98 (m, 2H), 6.94 (t, *J*=7.2 Hz, 1H), 5.04 (s, 2H), 4.05 (s, 2H), 2.34 (s, 6H)ppm. ^{13}C NMR (100 MHz, DMSO): δ 169.8, 167.4, 166.8, 149.2, 138.3, 135.5, 129.1, 128.3, 119.6, 116.5, 116.2, 112.7, 46.7, 35.9, 23.8 ppm. HRMS: m/z calcd for $C_{21}H_{22}N_3O_2S$ $[M + H]^+$ 380.1428, found 380.1441; $C_{21}H_{21}N_3NaO_2S$ $[M + Na]^+$ 402.1274, found 402.1258.

***N*-(4-((3-(2-((4,6-dimethylpyrimidin-2-yl)thio)acetamido)benzyl)oxy)phenyl)thiophene-2-carboxamide (28e)** First, *N*-(4-hydroxyphenyl)acetamide (1.05 equiv) react with 1-(bromomethyl)-3-nitrobenzene (1.0 equiv) to give the intermediate **26c** by the methods describe as prepared for **26b**; Second, to a solution of **26c** (1.0 equiv) in methanol (5ml/mmol) was added thionyl chloride (0.5ml/mmol) and the mixture was reflux for 0.5h. Then the reaction was concentrated in *vacuo* to remove the organic solvent and basified with $NaHCO_3$solution (pH 8) and extracted three times with ethyl acetate, the combined organic extracts were dried (Na_2SO_4), and concentrated under reduced pressure to give 4-((4-nitrobenzyl)oxy)aniline, which was taken up for the next step without any purification; Next, to a solution of 4-((4-nitrobenzyl)oxy)aniline (1.0 equiv) and Et_3N (3.0 equiv) in DCM (5ml/mmol) was added thiophene-2-carbonyl chloride (1.2 equiv) at 0°C, and the reaction was stirred for 2h. When TLC indicated that the reaction was finished, the reaction solution was

concentrated under reduced pressure and the crude product was purified by column chromatography (petroleum ether: EtOAc=4:1) to yield light yellow intermediate **26e**. Finally, using the key intermediate **26e**, the title compound **28e** was obtained by the general procedures as above, 18% for six steps, 97.0% HPLC purity. ^{1}H NMR (400 MHz, DMSO): δ 10.30(s, 1H), 10.13(s, 1H), 7.99 (d, *J*=3.2 Hz, 1H), 7.84 (dd, *J*=0.8 Hz, *J*=4.0 Hz, 1H), 7.71 (s, 1H), 7.63 (d, *J*=8.4 Hz, 2H), 7.55 (d, *J*=8.4 Hz, 1H), 7.34 (t, *J*=8.0 Hz, 1H), 7.22 (t, *J*=8.0 Hz, 1H), 7.14 (d, *J*=8.0 Hz, 1H), 7.02-6.97 (m, 3H), 5.08 (s, 2H), 4.06 (s, 2H), 2.36 (s, 6H)ppm. ^{13}C NMR (100 MHz, DMSO): δ 169.8, 167.4, 167.1, 160.0, 155.1, 140.7, 139.7, 138.3, 132.4, 132.0, 129.2, 128.5, 122.9, 122.5, 119.0, 118.5, 116.5, 115.3, 69.8, 36.0, 23.8 ppm. HRMS: m/z calcd for $C_{26}H_{25}N_4O_3S_2$ $[M + H]^+$ 505.1348, found 505.1369; $C_{26}H_{24}N_4NaO_3S_2$ $[M + Na]^+$ 527.1179, found 527.1181.

***N*-(3-(((1H-indazol-5-yl)oxy)methyl)phenyl)-2-((4,6-dimethylpyrimidin-2-yl)thio) acetamide (28k)** Using the commercially available1*H*-indazol-5-ol, the title compound **28k** was obtained using a method similar to that for **28a**, 17% for four steps, 96.6% HPLC purity. ^{1}H NMR (400 MHz, DMSO): δ 12.93 (s, 1H), 10.30 (s, 1H), 7.95 (s, 1H), 7.73 (s, 1H), 7.55 (d, *J*=8.0 Hz, 1H), 7.47 (d, *J*=8.8 Hz, 1H), 7.34 (t, *J*=8.0 Hz, 1H), 7.26 (s, 1H), 7.17 (d, *J*=8.0 Hz, 1H), 7.08 (dd, *J*=8.8 Hz, *J*=2.0 Hz, 1H), 6.96 (s, 1H), 5.11 (s, 2H), 4.05 (s, 2H), 2.32 (s, 6H)ppm. ^{13}C NMR (100 MHz, DMSO): δ 169.8, 167.4, 167.1, 153.2, 139.7, 138.5, 136.3, 133.3, 129.3, 123.5, 122.9, 118.9, 118.8, 118.5, 116.5, 111.6, 101.9, 70.1, 36.0, 23.8 ppm. HRMS: m/z calcd for $C_{22}H_{22}N_5O_2S$ $[M + H]^+$ 420.1489, found 420.1490; $C_{22}H_{21}N_5NaO_2S$ $[M + Na]^+$

442.1308, found 442.1313.

***N*-(3-(benzyloxy)phenyl)-2-((4,6-dimethylpyrimidin-2-yl)thio)acetamide (32)** A mixture of 3-aminophenol (**29**, 1.0 equiv), acetic anhydride (1.1 equiv), and triethylamine (3.0 equiv) in DCM (10ml/mmol)was stirred at room temperature overnight and the solvent was removed under reduced pressure. The solid residue was partitioned between EtOAc and saturated aqueous $NaHCO_3$, and the organic phase was dried (Na_2SO_4). The solvent was removed in *vacuo* to afford the*N*-(3-hydroxyphenyl)acetamide in 97% yield. Next, a reaction mixture of *N*-(3-hydroxyphenyl)acetamide (1.0 equiv) and *t*-BuOK (1.5 equiv) in DMF (5ml/mmol) was stirred for 0.5h at ambient temperature, and the resultant solution was treated with benzylbromide (1.1 equivl) and the reaction mixture was stirred overnight. After the reaction mixture was poured into a large amount of water (20ml/1ml DMF), the crude mixture was extracted with ethyl acetate (3×). Then, the combined organic layers were dried and concentrated. The residue was purified by column chromatography to give intermediate **31**, in 64% yield. Finally, using the key intermediate **31**, the title compound **32** was obtained by the general procedures as above, 23% for five steps, 97.3% HPLC purity. ^{1}H NMR (400 MHz, DMSO): δ 10.22(s, 1H),7.46-7.33 (m, 6H), 7.21 (d, *J*=8.4 Hz, 1H), 7.12 (d, *J*=8.4 Hz, 1H), 6.98 (s, 1H), 6.72 (dd, *J*=2.4 Hz, *J*=5.6 Hz, 1H), 5.07 (s, 2H), 4.04 (s, 2H), 2.34 (s, 6H) ppm. HRMS: m/z calcd for $C_{21}H_{22}N_3O_2S$ $[M + H]^+$ 380.1427, found 380.1441; $C_{21}H_{21}N_3NaO_2S$ $[M + Na]^+$ 402.1274, found 402.1259.

***N*-(4-(benzyloxy)phenyl)-2-((4,6-dimethylpyrimidin-2-yl)thio)acetamide (35)** A

reaction mixture of 1-fluoro-4-nitrobenzene (1.0 equiv), phenylmethanol (1.0 equiv) and KOH(3.0 equiv) in dioxane (6ml/mmol)was stirred at room temperature over night. Then the solvent was removed under reduced pressure and the solid residue was partitioned between water and EtOAc, and the organic phase was dried (Na_2SO_4), concentrated in *vacuo*. Next, the crude product was purified by column chromatography to yield1-(benzyloxy)-4-nitrobenzene. Finally, using 1-(benzyloxy)-4-nitrobenzene, the title compound was obtained by the general procedures described as above, 29% for four steps, 97.7% HPLC purity. ^{1}H NMR (400 MHz, DMSO): δ 10.10(s, 1H),7.49 (d, *J*=6.8 Hz, 2H), 7.46-7.44 (m, 2H), 7.41-7.38 (m, 2H), 7.35-7.31(m, 1H), 7.00-6.95 (m, 3H), 5.07 (s, 2H), 4.01 (s, 2H), 2.34 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO): δ 169.8, 167.4, 166.5, 154.8, 137.6, 132.9, 128.9, 128.2, 128.1, 121.1, 116.5, 115.3, 69.8, 35.8, 23.8 ppm. HRMS: m/z calcd for $C_{21}H_{22}N_3O_2S$ $[M + H]^+$ 380.1427, found 380.1442;$C_{21}H_{21}N_3NaO_2S$ $[M + Na]^+$ 402.1274, found 402.1260.

Molecular Docking Simulations

In this study, all the docking simulations were carried out using AutoDock Vina[35]. The crystal structure of SIRT2 complexed with SirReal2 (PDB ID: 4RMG)[29] was used as the docking template. All the water and solvant molecules as well as SirReal2 were removed, and NAD^+ was kept. Gasteiger-Marsili charges were added to protein model and non-polar hydrogens were merged onto their respective heavy atoms using AutoDockTools. The binding site is defined as a rectangular grid. The grid center was set as coordinates of [x, y, z=-16.0, -26.1, 11.8], and the grid size

was 22×25×23, which encompassed the entire SirReal2 binding site. The number of docking poses was set as 20, and other parameters were set as default. Docking results were viewed using PyMOL.

Cell Line and Cell Culture

Human MCF-7 breast carcinoma cells were purchased from American TypeCulture Collection (ATCC, Rockville, MD) and maintained in DMEM (corning, High Glucose) medium supplement with 10% FBS (cell box), 100 U/mlpenicillin(Sigma–Aldrich, USA)and 100 U/ml streptomycin(Sigma–Aldrich, USA).

Cell Growth Inhibition Assays

The MTT assay was used to detect the viability of cells. Human MCF-7 breast carcinoma cells were subcultured at 80~90% confluency and used within 10 passages. First, cells were collected and seeded in a 96-well plate, and the seeding densities were 3000 cells/well. Then, cells were grown in media (10% FBS) in 96-well plates for 24 h, at 37℃, 5% CO_2. Next, different concentrations of test compounds (stock solution in DMSO, diluted with media containing 10% FBS) were added to each well and were incubated for 120 h, at 37℃, 5% CO_2. Finally, concentration of DMSO per well was 1% v/v. At the end of the incubation period, 20μL of the MTT (5mg/ml, Sigma, USA) solution was added to each well. And then, the formazan crystals were dissolved with 50μL of acidified SDS (20%, w/v). Absorbance was determined at 570nm on Multiskan MK3 (Thermo Scientific, USA) the next day. Each assay was performed in three replicates and all experiments were repeated at least twice.

Western Blotting Analyses

The MCF-7 cells were plated to 6-well plates (Corning) at a density of 1*105 cells/well, and the experiments were initiated after 24 h. The cells were treated with vehicle (0.5% DMSO) or compound **28e** for 6 h. MCF-7 cells were then harvested, washed with ice-cold physiological saline, and lysed with RIPAlysis buffer (Beyotime, China) containing 1% cocktail (Sigma-Aldrich)[19,36]. Proteins were separated by gel electrophoresis on 10% SDS-PAGE gels and probed with specific antibodies used at a 1:1,000 dilution. Then anti-α-tubulin (T6074) and anti-α-tubulin acetylated (T6793) of mouse monoclonal antibodies were both purchased from Sigma Aldrich. The horse radish peroxidase-coupled secondary antibodies (Zhong Shan Golden Bridge Bio-technology, China) were used at 1:5,000.

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Appendix. Supplementary material

Supplementary data related to this article can be found online at doi:10.1000/j.ejmech.0000.00.000.

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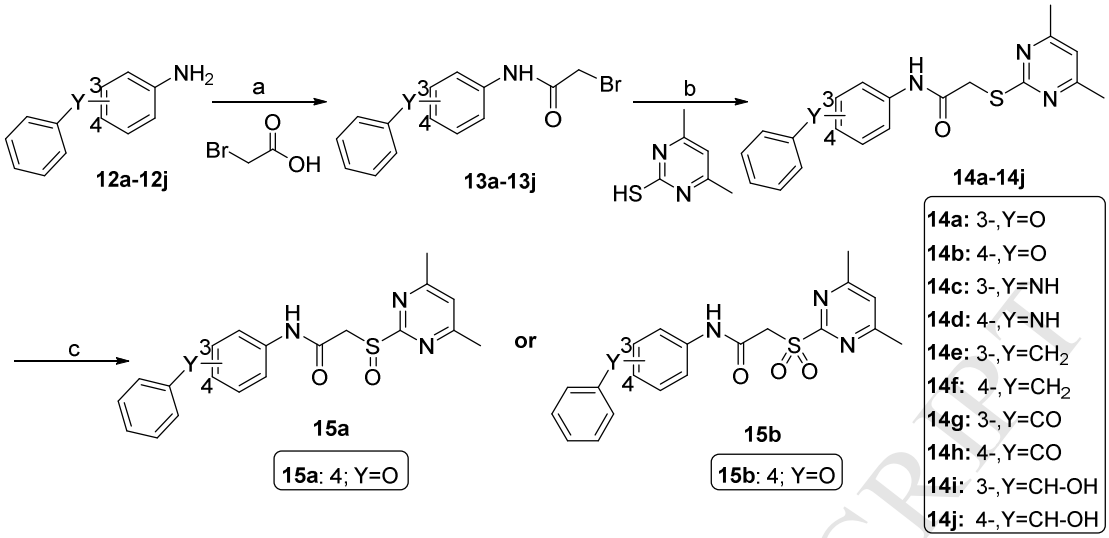
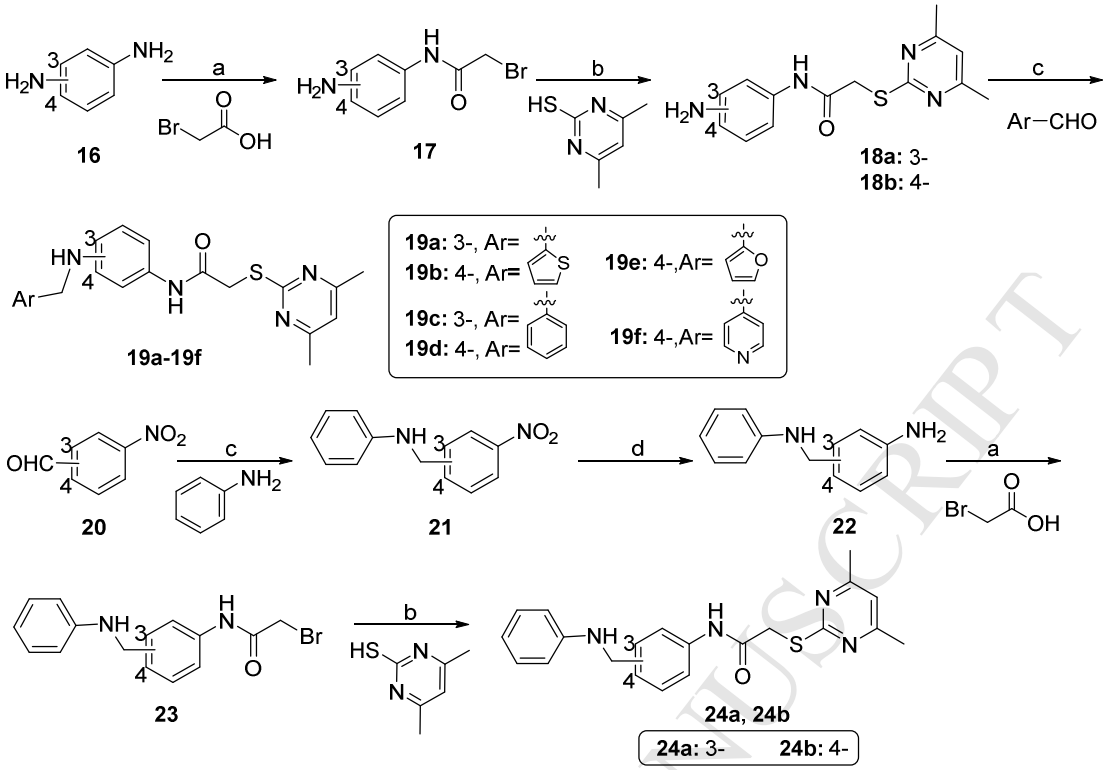
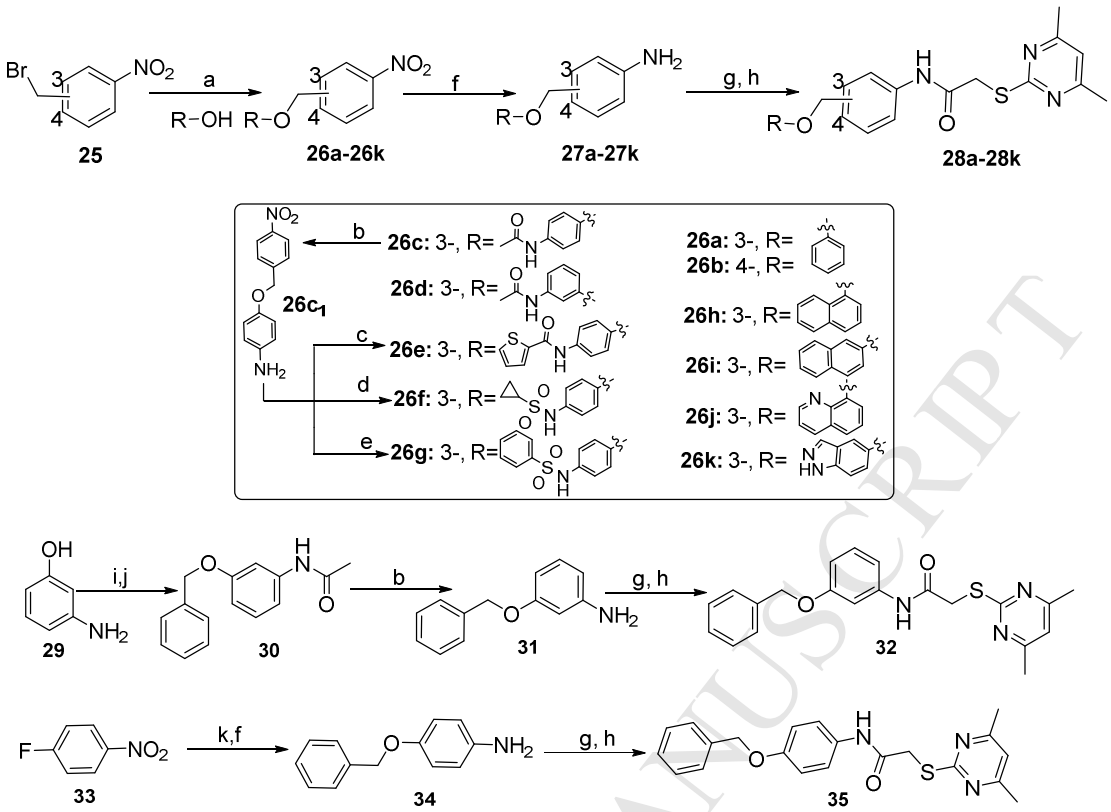


Scheme 1. Reagents and conditions: (a) HOBT, EDCI, DIPEA, DCM, RT, 12h, 62-93%; (b) *t*-BuOK, DMF, RT, 3-6h, 68-86%;(c) For **15a**: 1.0 equiv m-CPBA, DCM, RT, 1.0h, 66%; For **15b**: 3.0 equiv *m*-CPBA, DCM, RT, 3.5h, 78%.



Scheme 2. Reagents and conditions: (a) HOBT, EDCI, DIPEA, DCM, RT, 12h, 65-71%; (b) *t*-BuOK, DMF, RT, 3-6h, 78-80%; (c) Hantzsch Ester, molecular sieve,TFA, DCM, 45□, 12h, 61%-73%; (d)Fe, NH_4Cl, H_2O/EtOH=1:2, 85□, 0.5-1.0 h, 78-86% .



Scheme 3. Reagents and conditions: (a) KCO_3, DMF, RT, 3-4h, 80-82%; (b) $SOCl_2$, MeOH, 65□, 3h, 100%; (c) thiophene-2-carbonyl chloride, Et_3N, DCM, 0□, 2h, 70%; (d) cyclopropanesulfonyl chloride, Et_3N, DCM, 0□-RT, 5h, 62%; (e) benzenesulfonyl chloride, Et_3N, DCM, 0□-RT, 4h,76%; (f) Fe, NH_4Cl, H2O/EtOH=1:2, 85□, 0.5-1.0 h, 78-85%; (g) HOBT, EDCI, DIPEA, DCM, RT, 12h, 63-68%; (h) *t*-BuOK, DMF, RT, 3-6h, 69-75%; (i) acetic anhydride, Et_3N, DCM, RT, 12h, 97%; (j) (bromomethyl)benzene, t-BuOK, DMF, 12h, 64%; (k)phenylmethanol, Dioxane, KOH, RT, 12h, 93%.

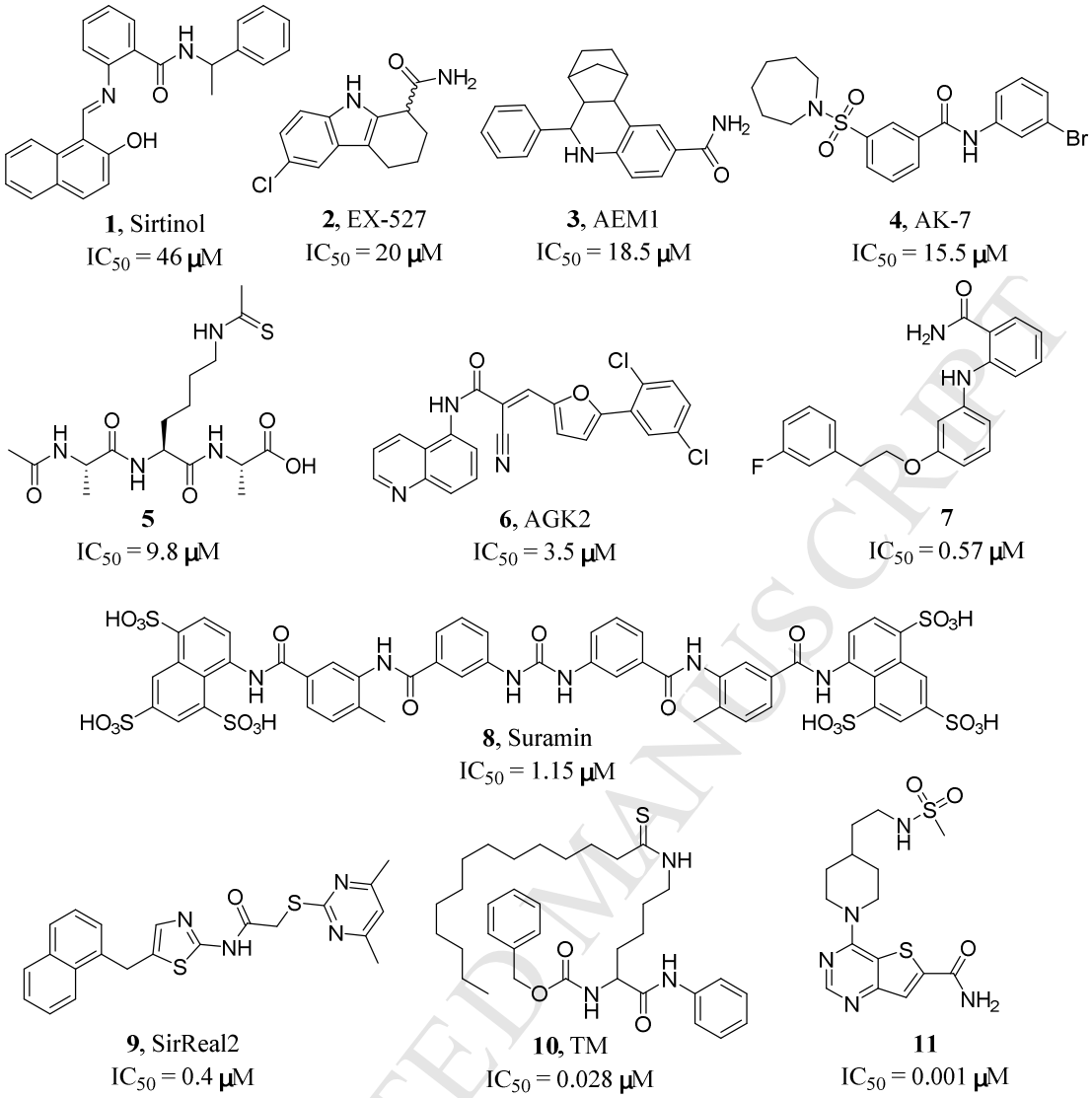


Figure 1. Chemical structures and inhibition potencies of reported SIRT2 inhibitors.

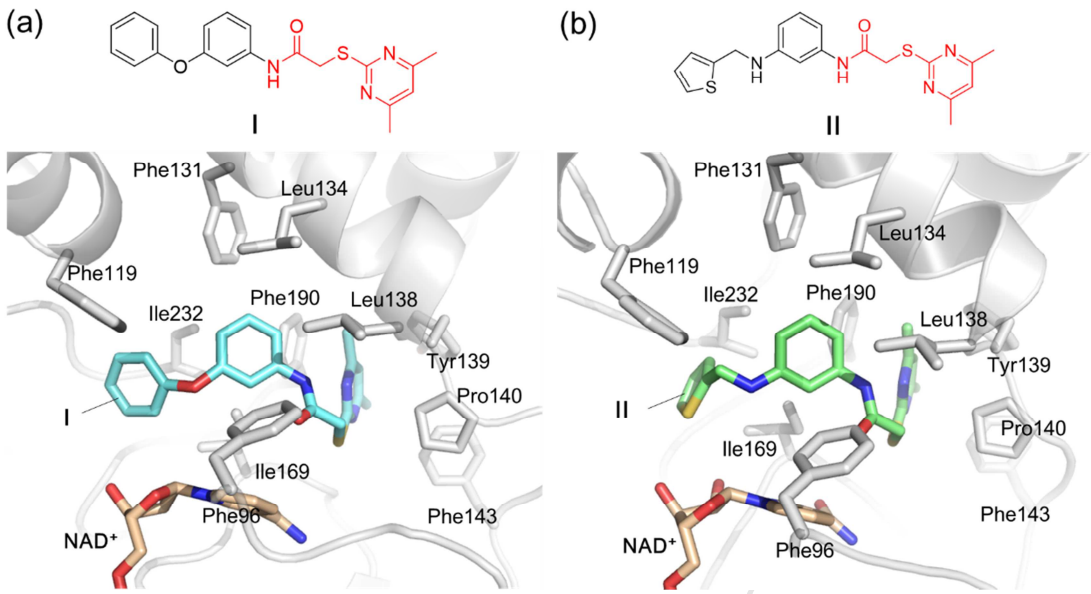


Figure 2. The two hit compounds **I** (a) and **II** (b) generated by LEADOPT and their predicted binding modes with SIRT2.**I**, **II**, NAD^+, and hydrophobic residues are shown in aquamarine, green, pink, and while sticks, respectively.

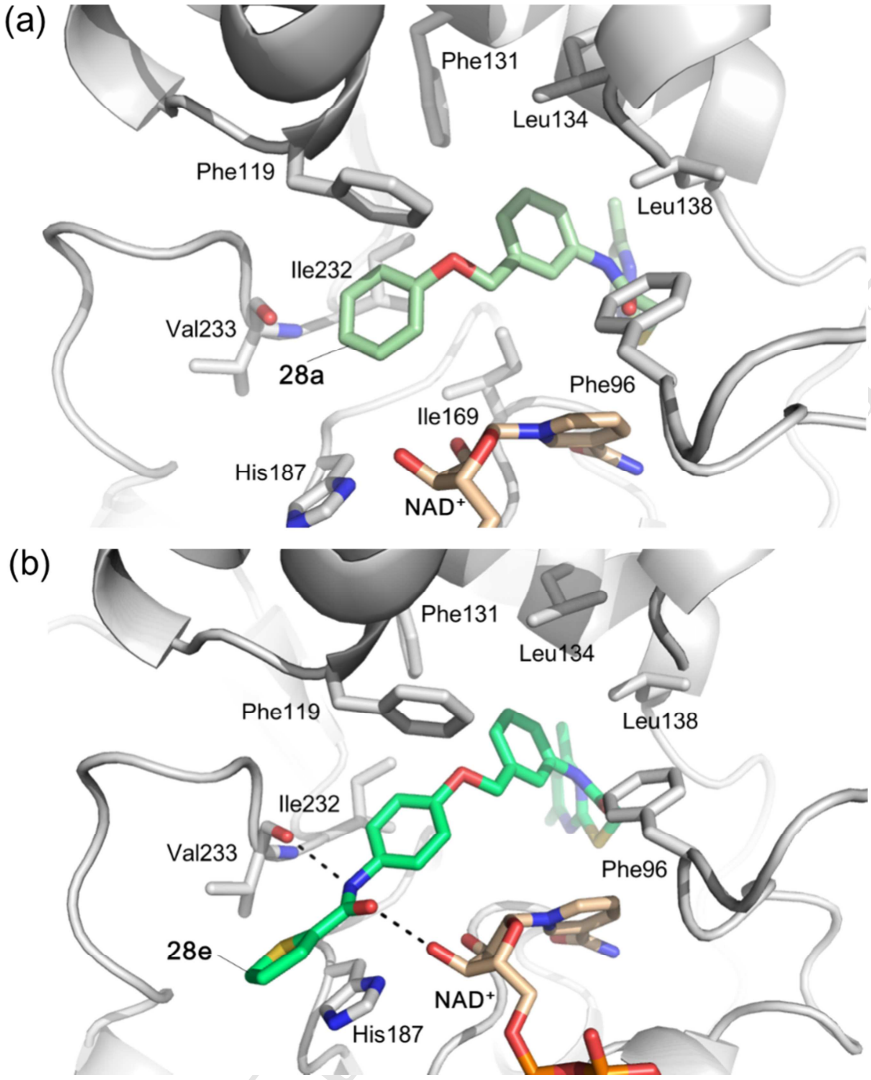


Figure 3. The predicted binding modes of **28a** (a) and **28e** (b) with SIRT2 using molecular docking simulations. **28a** appears positioned to make good hydrophobic interactions with Phe119, Ile232, Phe131, Leu134, Leu138, and Phe96; **28e** is likely to have additional hydrogen-bonding interactions with Val233 and pi-pi stacking interactions with His187.

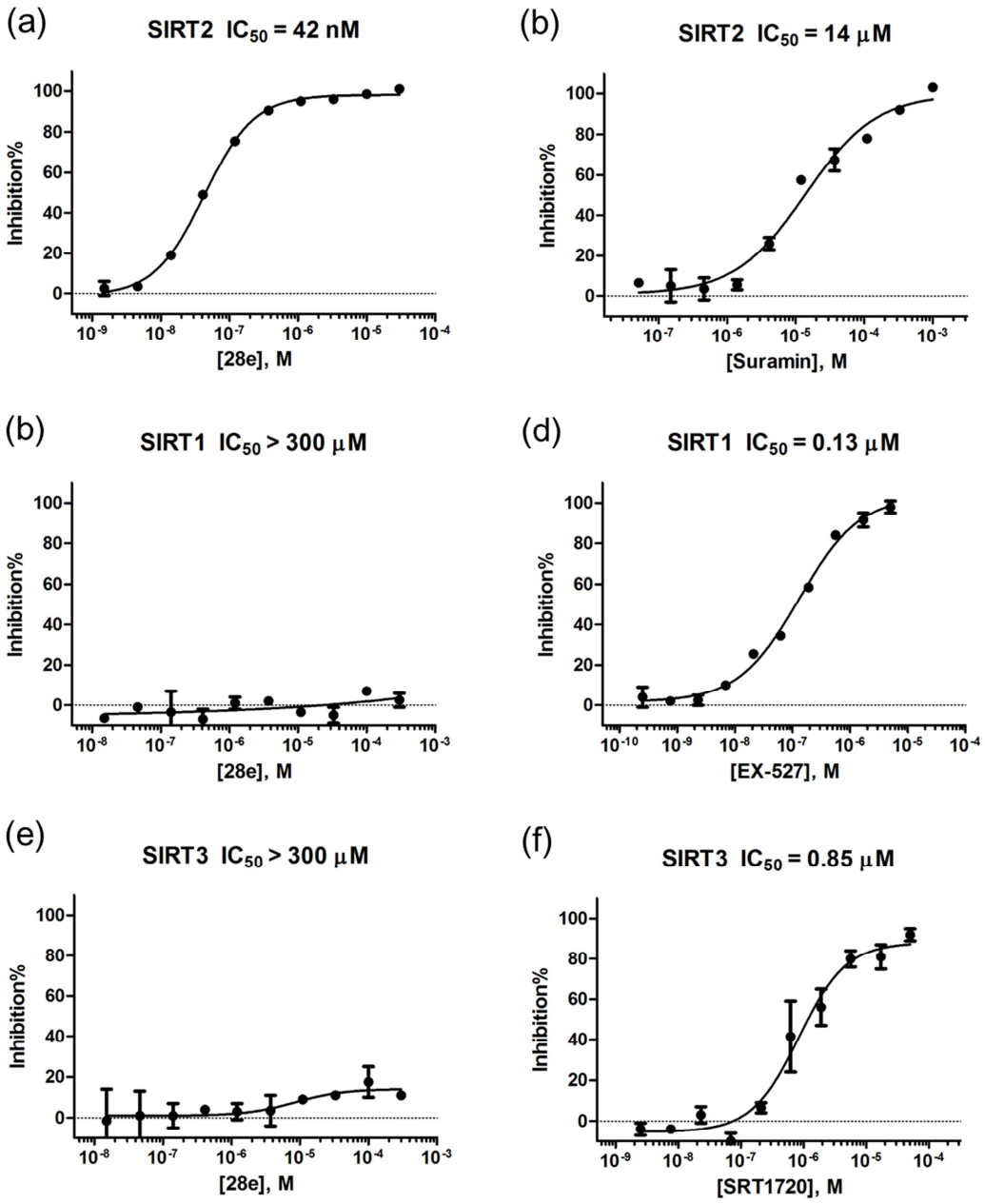


Figure 4. IC_{50} curves of **28e** with SIRT2 (a), Suramin with SIRT2 (b) **28e** with SIRT1 (c), EX-527 with SIRT1 (d), **28e** with SIRT3 (e), and SRT1720 with SIRT3 (f), indicating that **28e** has a substantial selectivity to SIRT2.

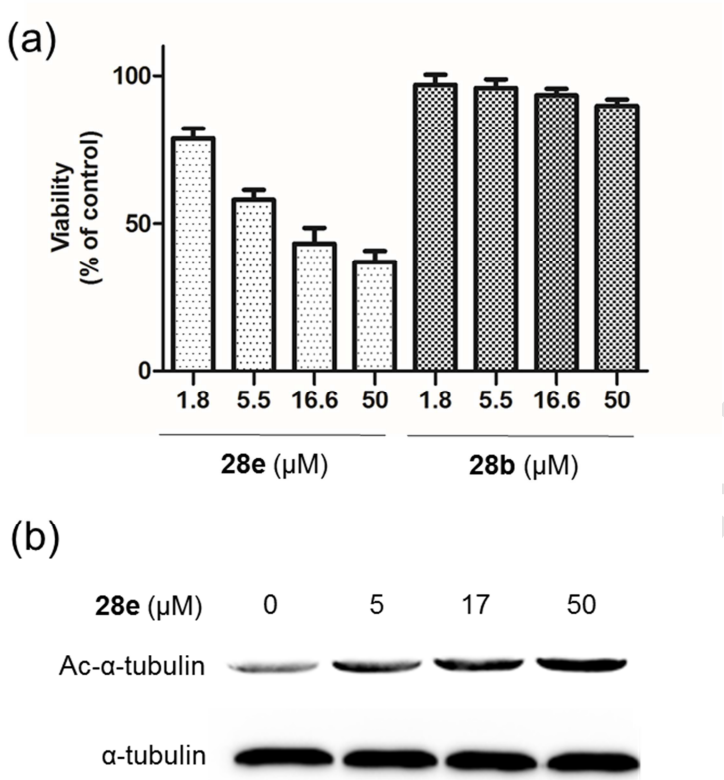
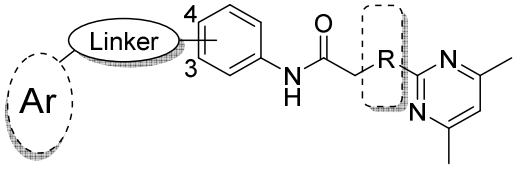


Figure 5. (a) Cellular inhibitory activities of compound **28e** against cancer line MCF-7 at different concentrations (1.8 μM, 5.5 μM, 16.6 μM, and 50 μM). We observed compound **28b**, which is structurally similar to **28e** but low potent inhibition to SIRT2, did not show obvious inhibition against MCF-7. (b) Immunoblot for acetyl-α-tubulin (K40) levels in MCF-7 cells treated with **28e** for 6 h, indicating **28e** can increase the acetylation of α-tubulin.

Table 1. Inhibitory activities of compounds with 3- or 4-substituentsat the phenyl of the 2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-phenylacetamidescaffold against human SIRT2.

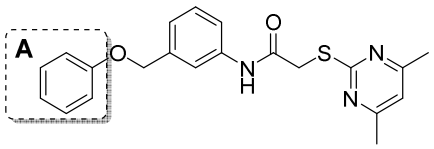


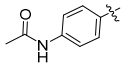
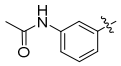
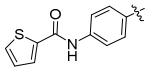
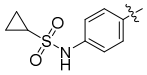
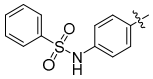
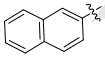
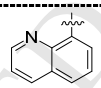
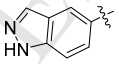
ID	Ar	L	Po	R	Inhibition%[a]	
					5μM	50μM
14a (I)			3		49±5	92±3
14b			4		42±4	78±3
14c			3		61±2	95±2
14d			4		19±3	72±5
14e			3		73±4	96±3
14f			4		70±3	74±6
14g			3		40±3	89±3
14h			4		22±5	74±2
14i			3		18±2	78±3
14j			4		26±3	75±3
15a			4		34±6	79±4
15b			4		-2±5	21±3
19a (II)			3		60±2	93±1
19b			4		15±2	70±3
19c			3		64±2	95±2
19d			4		9±2	55±1
19e			4		17±1	58±3

19f			4		10±3	43±2
24a			3		49±3	90±2
24b			4		20±4	72±3
32			3		59±1	92±2
35			4		40±2	62±3
28a			3		71±3	96±3
28b			4		1±1	24±1
Suramin					31±1	73±2

[a]Each compound was tested in triplicate; the data are presented as the mean ± SD (n=2).

Table 2. Inhibitory activities of 2-((4,6-dimethylpyrimidin-2-yl)thio)-*N*-(3-(**A**-oxyl methyl)phenyl)acetamide derivatives against human SIRT2.



ID	A	Inhibition%[a]	
		5μM	50μM
28c		62±1	92±3
28d		68±3	99±1
28e		98±1	99±2
28f		67±1	95±3
28g		73±1	87±1
28h		42±1	79±3
28i		59±2	84±2
28j		41±1	73±2
28k		57±2	91±2
Suramin		31±1	73±2

[a]Each compound was tested in triplicate; the data are presented as the mean ± SD (n=2).

Highlights

- Structure-based optimization led to the identification of new hit compounds for SIRT2
- SAR analyses revealed a number of new potent SIRT2 inhibitors
- The most potent inhibitor **28e** shows a significant selectivity to SIRT2 over SIRT1 and SIRT3.
- **28e** displays inhibition against cancer line MCF-7 by suppressing SIRT2.