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PII: S0960-894X(15)00400-X
DOI: http://dx.doi.org/10.1016/j.bmcl.2015.04.068
Reference: BMCL 22651

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 15 February 2015
Revised Date: 15 April 2015
Accepted Date: 20 April 2015

Please cite this article as: Therrien, E., Larouche, G., Nguyen, N., Rahil, J., Lemieux, A-M., Li, Z., Fournel, M., Yan, T.P., Landry, A-J., Lefebvre, S., Wang, J.J., MacBeth, K., Heise, C., Nguyen, A., Besterman, J.M., Déziel, R., Wahhab, A., Discovery of Bicyclic Pyrazoles as Class III Histone Deacetylase SIRT1 and SIRT2 Inhibitors, *Bioorganic & Medicinal Chemistry Letters* (2015), doi: http://dx.doi.org/10.1016/j.bmcl.2015.04.068

Discovery of Bicyclic Pyrazoles as Class III Histone Deacetylase SIRT1 and SIRT2 Inhibitors

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Received Month XX, 2015; Accepted Month XX, 2015 [BMCL RECEIPT]

This manuscript is dedicated to Professor Stephen Hanessian on the occasion of his 80th birthday.

Abstract—A series of bicyclic pyrazole carboxamides was synthesized and tested for inhibitory activity against the class III deacetylase sirtuin enzymes. Moderate to low micromolar inhibitory activities were obtained against SIRT1 and SIRT2. These bicyclic pyrazole compounds represent a new class of sirtuin inhibitors with a preference for SIRT1 over SIRT2. **Keywords**: Sirtuin; SIRT; inhibitor; pyrazole; docking.

The histone deacetylase (HDAC) family of enzymes is known to catalyze the removal of an acetyl group from lysine residues at the N-terminal tails of histone proteins. Class I (HDAC1-3 and 8), class IIa (HDAC4-7 and 9), and class IIb (HDAC6 and 10) HDACs remove acetyl groups through a zinc-mediated hydrolysis.[1,2] However, class III, also known as sirtuins are NAD-dependent histone deacetylases that catalyze the deacetylation of acetylated lysine resulting in the formation of 2'- and 3'-*O*-acetyl-ADP-ribose[3,4] and free nicotinamide. Silent information regulator 2 (Sir2) family of proteins (sirtuins) comprises five homologues in yeast (ySir2 and HST1-4) and seven in humans (SIRT1-7).[5-7]

SIRT1, the most studied of all sirtuins, has been implicated in several cellular processes such as metabolism, fat mobilization, differentiation, and muscle development.[8-10] SIRT1 has also been demonstrated to deacetylate different transcription factors such as p53, the FOXO family, and NF-κB.[11-13] Activators of SIRT1, such as resveratrol and a structurally unrelated small molecule SRT2104, are also very well studied and show promising results in clinical trials.[14,15] SIRT2 was originally linked to age-associated diseases such as Huntington's disease, Alzheimer's disease, and diabetes,[16,17] mainly due to its cytosolic localization. Recently, SIRT1 and SIRT2 have been considered as targets for cancer chemotherapy.[18-22]

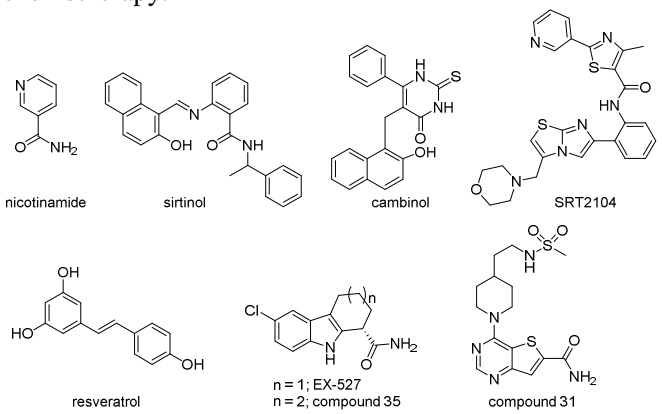

Figure 1. Known sirtuin inhibitors and activators.

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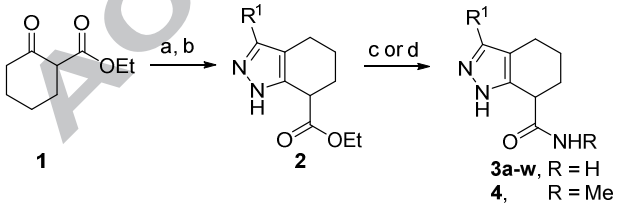
Among the few known inhibitors of sirtuins[23-25] (Fig. 1), only a limited number exhibit inhibitory activity against human subtypes. Besides the common and unselective inhibitor nicotinamide,[26] sirtinol[27] was one of the first synthetic small molecule inhibitors of SIRT1. Although sirtinol is a micromolar inhibitor of yeast Sir2p enzyme and human SIRT2, it is a weak inhibitor of SIRT1.[28,29] The sirtuin inhibitor cambinol, which was identified as active against both human SIRT1 and SIRT2 enzymes, showed antitumor activity *in vitro* and in mouse xenograft models.[30]

Several indoles were identified from a high-throughput screen as selective inhibitors of SIRT1.[31] The most potent analogue, EX-527 has a reported IC_{50} of 0.1 μM against SIRT1 and inhibits the deacetylation of p53 at a concentration of 1 μM.[31] More recently, low nanomolar pan-inhibitors of SIRT1, SIRT2, and SIRT3 (e.g. compound 31) were discovered through affinity screening using encoded library technology (ELT).[32]

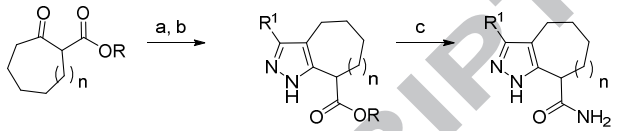
At the time of this investigation, we envisaged EX-527 as a starting point for the design of novel small molecule sirtuin inhibitors. Our initial efforts were based on identification of a replacement for the central core of the molecule. Hence, we utilized a pyrazole scaffold in lieu of an indole. We herein describe the synthesis and biological evaluation of an unprecedented series of bicyclic pyrazole derivatives as SIRT1 and SIRT2 inhibitors.

The synthesis of these pyrazoles was achieved via the dianion of the commercially available ethyl 2-oxocyclohexanecarboxylate **1** which was acylated with either the desired acyl chloride or the methyl carboxylate and the resulting 1,3-diketone intermediate[33] was reacted with hydrazine hydrate in THF to provide the tetrahydroindazole **2** (Scheme 1). The ethyl ester **2** was then converted to the carboxamide **3** in the presence of a 7N solution of ammonia in methanol upon heating in a sealed tube. The methyl amide **4** was obtained from ester **2** using a solution of methylamine in methanol.



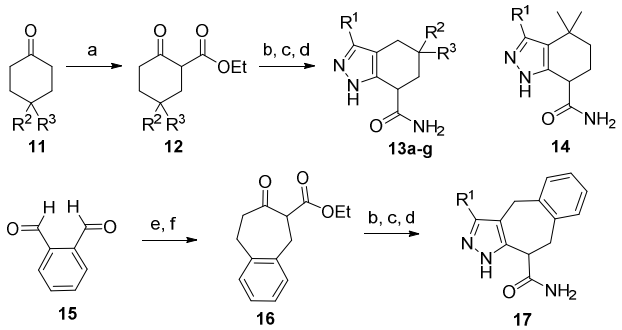
Scheme 1. Reagents and conditions: (a) NaH, n-BuLi, THF 0 °C then R^1COCl or R^1CO_2Me; (b) $NH_2NH_2.H_2O$, THF; (c) NH_3 7N in MeOH, 95 °C (sealed tube); (d) $MeNH_2$, MeOH, 95 °C (sealed tube).

In order to further explore the chemical space of the active site of the enzyme, we modified the ring size on the bicyclic pyrazole scaffold. Thus, the 7- and 8-membered ring analogues were synthesized (Scheme 2). Acylation of the bis-enolate of methyl 2-oxocycloheptanecarboxylate **5** and subsequent treatment with hydrazine hydrate yielded the corresponding hexahydrocycloheptapyrazole **7**. Conversion of the methyl carboxylate **7** to the carboxamide **9** was achieved under the usual conditions. Similarly, the hexahydrocyclooctapyrazole analogue **10** was also synthesized from keto-ester **6**.



Scheme 2. Reagents and conditions: (a) NaH, n-BuLi, THF 0 °C then 4-Cl-PhCOCl; (b) $NH_2NH_2.H_2O$, THF; (c) NH_3 7N in MeOH, 95 °C (sealed tube).

Ring modifications were also expanded to include various substitutions. Thus, mono- and di-substituted cyclohexanones **11** were converted to their corresponding keto-ester analogues **12** (Scheme 3). Acylation of the dianion derived from **12** with a selection of acyl chlorides or methyl carboxylates provided the 1,3-diketone intermediates (not shown in Scheme 3) which were further transformed to pyrazoles **13a-g** using the method described above. The gem-dimethyl regioisomer **14** was also synthesized based on this procedure starting from 3,3-dimethylcyclohexanone. The tricyclic analogue **17** was made from 4,5-benzo-cycloheptanone carboxylate **16** which was synthesized as described in the literature.[34]



Scheme 3. Reagents and conditions: (a) NaH, KH, diethyl carbonate, THF, 65 °C; (b) NaH, n-BuLi, THF 0 °C then R^1COCl or R^1CO_2Me; (c) $NH_2NH_2.H_2O$, THF (d) NH_3 7N in MeOH, 95 °C (sealed tube); (e) Ethyl 3-oxo-4-(triphenylphosphoranylidene)butyrate, THF, 120 °C; (f) H_2, Pd/C, EtOH.

The inhibitory activities of SIRT1 and SIRT2 for different aryl and heteroaryl substituted bicyclic pyrazoles, compounds **3a-w**, are presented in Table 1. The inhibitory activities were tested in a fluorescence-based assay using Cbz-Lys(Ac)-AMC as a substrate.[35] Attempts to separate the two enantiomers of compound **3b** by chiral HPLC[36] failed to provide the required separation level to yield optically pure material. Therefore, the compounds were tested as

their racemic mixtures of stereoisomers. In general, the bicyclic pyrazoles displayed a moderate degree of selectivity for SIRT1 over SIRT2 and did not show any inhibition of class I or class II HDACs (data not shown).[37]

The unsubstituted phenyl ring (**3a**) provided a weak inhibitor of SIRT1 (showing only partial inhibition at 20 μM). Systematic variation of the position of the chlorine on the phenyl ring resulted in the 4-chlorophenyl pyrazole **3b** being the most active among analogues **3b-d** showing IC_{50} of 1.0 μM for SIRT1. Equipotent inhibitor was obtained with the 3,4-dichlorophenyl analogue **3e**, whereas the regioisomer **3f** was devoid of activity. The replacement of the 3-chlorine of **3e** with fluorine gave a slightly weaker inhibitor **3g**. Exchange of the para-chlorine on the phenyl ring with different groups (**3h-l**) reduced considerably the inhibitory activity against SIRT1. Both the N-methylamide **4b** and the ethyl carboxylate **2g** showed no inhibition at 20 μM, indicating an essential requirement for the primary carboxamide.

Table 1. SIRT inhibitory activities for different aryl and heteroaryl substituted bicyclic pyrazoles.

Compounds	R^1	SIRT1 IC_{50} μM (% inh @ 20 μM)[a,b]	SIRT2 IC_{50} μM (% inh @ 20 μM)[a,b]
EX-527[c]	-	0.5	6.5
3a	Phenyl	(44%)	NI
3b	4-Chlorophenyl	1.0	(43%)
4b	4-Chlorophenyl	NI	NI
3c	3-Chrorophenyl	(27%)	NI
3d	2-Chrorophenyl	NI	(11%)
3e	3,4-Dichlorophenyl	1.2	14.8
3f	2,4-Dichlorophenyl	(24%)	NI
3g	4-Chloro-3-fluorophenyl	2.6	(44%)
2g	4-Chloro-3-fluorophenyl	NI	NI
3h	4-Bromophenyl	1.9	(47%)
3i	4-Fluorophenyl	(42%)	NI
3j	4-Methylphenyl	6.2	(36%)
3k	4-Methoxyphenyl	NI	NI
3l	4-Trifluoromethylphenyl	NI	NI
3m	2-Thiophene	(47%)	NI
3n	4-Bromo-2-thiophene	11.0	NI
3o	2-Benzothiophene	1.7	5.3
3p	2-Benzofuran	3.1	9.0
3q	2-Benzothiazole	2.2	8.3
3r	N-Methyl-2-indole	2.7	7.8
3s	2-Indole	(46%)	(43%)
3t	5-Chloro-N-methyl-2-indole	(31%)	(51%)
3u	5-Bromo-2-benzothiophene	(38%)	(33%)
3v	N-Methyl-3-indole	NI	NI
3w	3-Benzothiophene	(48%)	(44%)

[a]Values are means of three experiments. All compounds are racemic. [b]NI = no inhibition (less than 20% at 20 μM). [c]Mixture of diastereoisomers.

Replacement of the 4-chlorophenyl substituent with different heteroaryl moieties led to bicyclic pyrazoles **3m-w**. The 2-thiophene **3m** gave a compound with similar activity to that of phenyl **3a**. Analogues bearing fused bicyclic heteroaryl rings attached at the 2-position such as 2-benzothiophene **3o**, 2-benzofuran **3p**, 2-benzothiazole **3q** and N-methyl-2-indole **3r** proved to be tolerated replacements of the 4-chlorophenyl group. Although these compounds did not show increased enzymatic inhibitory activities against SIRT1 compared to the 4-chlorophenyl analogue **3b**, they showed moderate activity against SIRT2 with IC_{50} of 5.3-9.0 μM (**3o-r**). However, further substitutions (**3t-u**) and different attachment points (**3v-w**) led to weaker inhibitors against both enzymes.

Table 2 lists SIRT inhibitory activities for different ring modifications of aryl and heteroaryl bicyclic pyrazoles. The seven-membered ring compound **9** was 2-fold less potent against SIRT1 than the corresponding tetrahydroindazole **3b**, whereas the eight-membered ring analogue **10** showed no inhibitory activity. All attempts to prepare the unprecedented five-membered ring derivative failed. Further modifications on the ring led to compound **13a** with increased activity against both SIRT enzymes. Unlike modifications of pyrazoles **3**, every variation of the R^1 group of **13a** (compounds **13b-e**) was detrimental to the activity against both enzymes. Mono-phenyl **13f** and fused benzyl compound **17** showed only partial inhibition while spiro ketal **13g** and regioisomer gem-dimethyl analogue **14** were devoid of activity.

Table 2. SIRT inhibitory activities for different ring modifications of aryl and heteroaryl bicyclic pyrazoles.

Compounds	R^1	R^2	R^3	SIRT1 IC_{50} μM (% inh @ 20 μM)[a,b]	SIRT2 IC_{50} μM (% inh @ 20 μM)[a,b]
9	4-Chlorophenyl	-	-	2.1	6.8
10	4-Chlorophenyl	-	-	(28%)	(22%)
13a	4-Chlorophenyl	Me	Me	0.8	1.7
13b	3,4-Dichlorophenyl	Me	Me	(23%)	(28%)
13c	4-Bromophenyl	Me	Me	(56%)	(42%)
13d	N-Methyl-2-indole	Me	Me	NI	NI
13e	2-Benzothiophene	Me	Me	(53%)	(67%)
13f[c]	4-Chlorophenyl	Ph	H	(59%)	(62%)
13g	4-Chlorophenyl			NI	NI
14	4-Chlorophenyl	-	-	NI	NI
17	4-Chlorophenyl	-	-	(42%)	(50%)

[a]Values are means of three experiments. All compounds are racemic. [b]NI = no inhibition (less than 20% at 20 μM). [c]Mixture of diastereoisomers.

In addition to the inhibitory activity of both SIRT1 and SIRT2, we evaluated the ability of the bicyclic pyrazoles to inhibit the endogenous deacetylase activity of p53 in Namalwa cells (human, blood, lymphoma, Burkitt) using an ELISA-based assay (Table 3).[38] The compounds were ranked by their IC_{2xB}, which is defined as the inhibitor concentration needed to enhance p53 deacetylation to twice the basal level. In general, compounds that showed low

micromolar activity against SIRT1 enzyme *in vitro* inhibited p53 deacetylation in the cells. Compounds **3b**, **3e**, **3h**, **3q**, **9**, and **13a** showed p53 deacetylation with IC_{2xB} from 0.5 to 1.5 µM in accord with their enzymatic potency. Although a correlation was observed between p53 deacetylation and SIRT1 inhibition, further investigation is required to help substantiate that the cellular effects are due to Sirt1 inhibiton.

Table 3. SIRT1 enzymatic activity and *in vitro* p53 deacetylation inhibitory activity of bicyclic pyrazoles.[a]

Compounds	p53 deacetylation, IC_{2xB}, µM[c]	SIRT1 IC_{50}, µM
EX-527[b]	0.6	0.5
3b	0.5	1.0
3e	1.2	1.2
3h	1.5	1.9
3q	0.9	2.2
9	0.8	2.1
13a	1.2	0.8

[a]Values are means of three experiments. [b]Inhibitory activity was determined in our laboratories. [c]This is an in-house method developed to rank the ability of our SIRT1 inhibitors to inhibit p53 deacetylation. The value reported is the measured concentration at twice the basal level (basal: the lowest detected fluorescence value in Rfu).

Preliminary investigation of the pharmacokinetic profile of **3b** and **13a**, indicated that both compounds were stable in mouse plasma. In rats, **3b** had a clearance of 1.8 (L/h)/kg and a half-life of 1.1 hours. The bicyclic pyrazole **13a** had a lower clearance (0.3 (L/h)/kg) and a longer half-life (5.1 hours). Both **3b** and **13a** had moderate oral bioavailability of 20% and 23%, respectively.

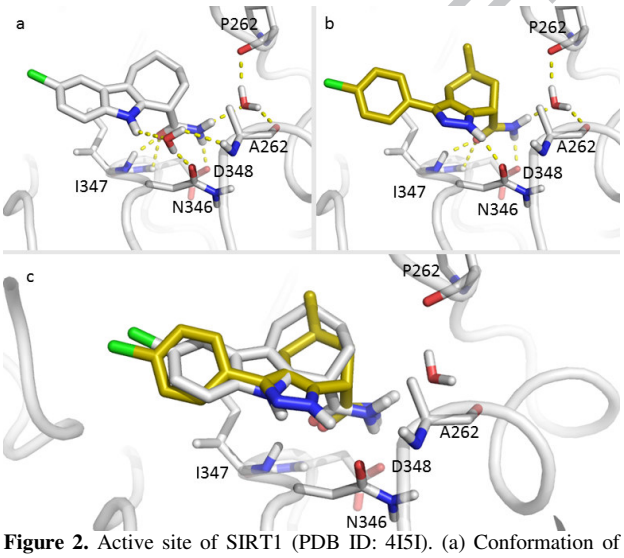


Figure 2. Active site of SIRT1 (PDB ID: 4I5I). (a) Conformation of compound 35 in the X-ray structure; (b) Docked conformation of **13a**; (c) Overlay of the docked conformation of **13a** (yellow) and compound 35 (gray).

Docking analysis in conjunction with the results of the *in vitro* SAR illustrated the importance of key interactions of the inhibitors with the enzyme. Figure 2 shows an overlay of compound **13a** with the co-crystallized EX-527 analogue (compound 35, PDB ID: 4I5I) obtained from docking experiments using the automated docking software FITTED.[39,40] All docked inhibitors showed a similar binding mode when the (R)-isomers were considered.

Key interactions of the inhibitor with the active site of the enzyme are the hydrogen bonds of the carboxamide with residues Asp348 and Ile347, the pyrazole N-H with the residue Asn346, and the hydrophobic contacts of the aryl group with amino acid residues Phe273, Phe297, Ile279, Ile316, and Ile347 (not shown). One of the two bridging water molecules was displaced during the docking[41] to accommodate the pyrazole in **13a** which occupies a slightly shifted position as compared with compound 35 in the X-ray structure, although a very well organized hydrogen bond network was conserved.

In conclusion, we have discovered a new class of sirtuin inhibitors based on the bicyclic pyrazole scaffold. Structure-activity relationships led to the identification of a few compounds that are low micromolar inhibitors of SIRT1. However, there was little tolerance for substitution both on the phenyl ring and on the tetrahydrocyclohexane moiety. The simplicity of these molecules and their relative ease of synthesis should lead to further optimization of this new class of sirtuin inhibitors.

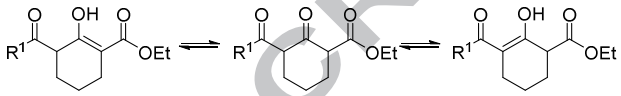
Acknowledgments

The authors like to thank Dr. Arkadii Vaisburg for his comments and corrections to this manuscript.

References and notes

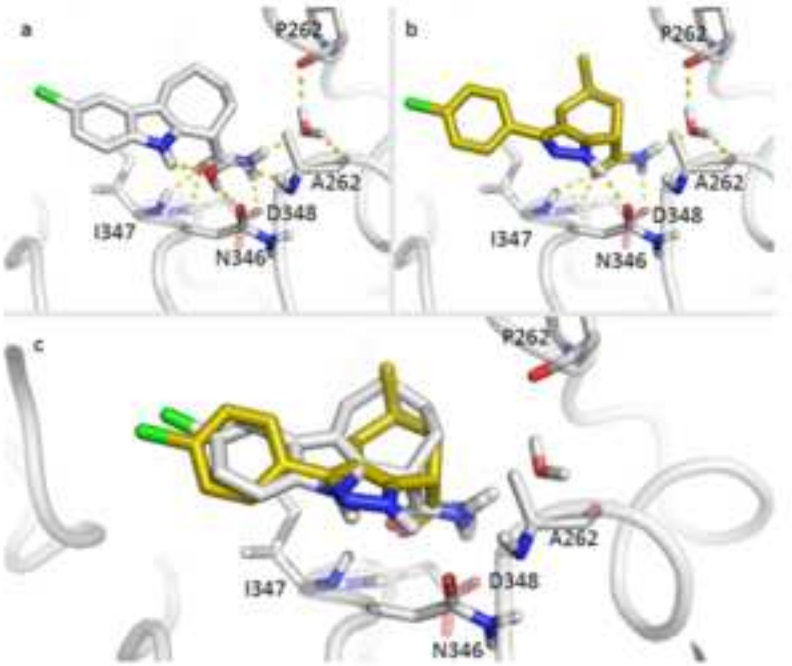
1. Khochbin, S.; Verdel, A.; Lemercier, C.; Seigneurin-Berny, D. *Curr. Opin. Genet. Dev.* **2001,** *11*, 162.
2. Sauve, A. A.; Wolberger, C.; Schramm, V. L.; Boeke, J. D. *Annu. Rev. Biochem.* **2006,** *75*, 435.
3. Imai, S.; Armstrong, C. M.; Kaeberlein, M.; Guarente, L. *Nature* **2000,** *403*, 795.
4. Tanner, K. G.; Landry, J.; Sternglanz, R.; Denu, J. M. *Proc. Natl. Acad. Sci. U. S. A.* **2000,** *97*, 14178.
5. Frye, R. A. *Biochem. Biophys. Res. Commun.* **1999,** *260*, 273.
6. Frye, R. A. *Biochem. Biophys. Res. Commun.* **2000,** *273*, 793.
7. North, B. J.; Verdin, E. *Genome Biol* **2004,** *5*, 224.
8. Picard, F.; Kurtev, M.; Chung, N.; Topark-Ngarm, A.; Senawong, T.; Machado De Oliveira, R.; Leid, M.; McBurney, M. W.; Guarente, L. *Nature* **2004,** *429*, 771.
9. Fulco, M.; Schiltz, R. L.; Iezzi, S.; King, M. T.; Zhao, P.; Kashiwaya, Y.; Hoffman, E.; Veech, R. L.; Sartorelli, V. *Mol. Cell* **2003,** *12*, 51.
10. Guarente, L. *Genes Dev.* **2000,** *14*, 1021.

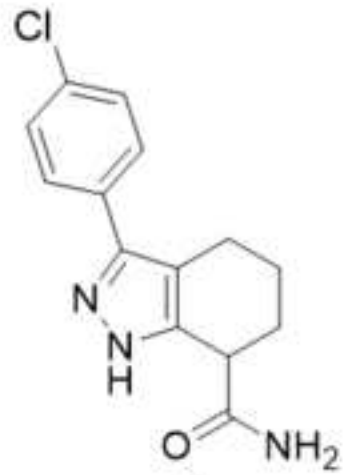
11. Luo, J.; Nikolaev, A. Y.; Imai, S.; Chen, D.; Su, F.; Shiloh, A.; Guarente, L.; Gu, W. *Cell* **2001,** *107*, 137.
12. Motta, M. C.; Divecha, N.; Lemieux, M.; Kamel, C.; Chen, D.; Gu, W.; Bultsma, Y.; McBurney, M.; Guarente, L. *Cell* **2004,** *116*, 551.
13. Yeung, F.; Hoberg, J. E.; Ramsey, C. S.; Keller, M. D.; Jones, D. R.; Frye, R. A.; Mayo, M. W. *EMBO J.* **2004,** *23*, 2369.
14. Kaeberlein, M.; McDonagh, T.; Heltweg, B.; Hixon, J.; Westman, E. A.; Caldwell, S. D.; Napper, A.; Curtis, R.; DiStefano, P. S.; Fields, S.; Bedalov, A.; Kennedy, B. K. *J. Biol. Chem.* **2005,** *280*, 17038.
15. Milne, J. C.; Denu, J. M. *Curr. Opin. Chem. Biol.* **2008,** *12*, 11.
16. Aljada, A.; Dong, L.; Mousa, S. A. *Curr Opin Investig Drugs* **2010,** *11*, 1158.
17. Kitada, M.; Kume, S.; Kanasaki, K.; Takeda-Watanabe, A.; Koya, D. *Curr. Drug Targets* **2013,** *14*, 622.
18. Hoffmann, G.; Breitenbucher, F.; Schuler, M.; Ehrenhofer-Murray, A. E. *J. Biol. Chem.* **2014,** *289*, 5208.
19. Rotili, D.; Tarantino, D.; Nebbioso, A.; Paolini, C.; Huidobro, C.; Lara, E.; Mellini, P.; Lenoci, A.; Pezzi, R.; Botta, G.; Lahtela-Kakkonen, M.; Poso, A.; Steinkuhler, C.; Gallinari, P.; De Maria, R.; Fraga, M.; Esteller, M.; Altucci, L.; Mai, A. *J. Med. Chem.* **2012,** *55*, 10937.
20. Mahajan, S. S.; Scian, M.; Sripathy, S.; Posakony, J.; Lao, U.; Loe, T. K.; Leko, V.; Thalhofer, A.; Schuler, A. D.; Bedalov, A.; Simon, J. A. *J. Med. Chem.* **2014,** *57*, 3283.
21. Hu, J.; Jing, H.; Lin, H. *Future Med Chem* **2014,** *6*, 945.
22. Bosch-Presegue, L.; Vaquero, A. *Genes Cancer* **2011,** *2*, 648.
23. Neugebauer, R. C.; Uchiechowska, U.; Meier, R.; Hruby, H.; Valkov, V.; Verdin, E.; Sippl, W.; Jung, M. *J. Med. Chem.* **2008,** *51*, 1203.
24. Huhtiniemi, T.; Suuronen, T.; Rinne, V. M.; Wittekindt, C.; Lahtela-Kakkonen, M.; Jarho, E.; Wallen, E. A.; Salminen, A.; Poso, A.; Leppanen, J. *J. Med. Chem.* **2008,** *51*, 4377.
25. Neugebauer, R. C.; Sippl, W.; Jung, M. *Curr. Pharm. Des.* **2008,** *14*, 562.
26. Sauve, A. A.; Moir, R. D.; Schramm, V. L.; Willis, I. M. *Mol. Cell* **2005,** *17*, 595.
27. Grozinger, C. M.; Chao, E. D.; Blackwell, H. E.; Moazed, D.; Schreiber, S. L. *J. Biol. Chem.* **2001,** *276*, 38837.
28. Mai, A.; Massa, S.; Lavu, S.; Pezzi, R.; Simeoni, S.; Ragno, R.; Mariotti, F. R.; Chiani, F.; Camilloni, G.; Sinclair, D. A. *J. Med. Chem.* **2005,** *48*, 7789.
29. Pasco, M. Y.; Rotili, D.; Altucci, L.; Farina, F.; Rouleau, G. A.; Mai, A.; Neri, C. *J. Med. Chem.* **2010,** *53*, 1407.
30. Heltweg, B.; Gatbonton, T.; Schuler, A. D.; Posakony, J.; Li, H.; Goehle, S.; Kollipara, R.; Depinho, R. A.; Gu, Y.; Simon, J. A.; Bedalov, A. *Cancer Res.* **2006,** *66*, 4368.
31. Napper, A. D.; Hixon, J.; McDonagh, T.; Keavey, K.; Pons, J. F.; Barker, J.; Yau, W. T.; Amouzegh, P.; Flegg, A.; Hamelin, E.; Thomas, R. J.; Kates, M.; Jones, S.; Navia, M. A.; Saunders, J. O.; DiStefano, P. S.; Curtis, R. *J. Med. Chem.* **2005,** *48*, 8045.
32. Disch, J. S.; Evindar, G.; Chiu, C. H.; Blum, C. A.; Dai, H.; Jin, L.; Schuman, E.; Lind, K. E.; Belyanskaya, S. L.; Deng, J.; Coppo, F.; Aquilani, L.; Graybill, T. L.; Cuozzo, J. W.; Lavu, S.; Mao, C.; Vlasuk, G. P.; Perni, R. B. *J. Med. Chem.* **2013,** *56*, 3666.
33. The acylated 1,3-diketone intermediate has the following structure:



34. Albrecht, U.; Nguyen, T. H.; Langer, P. *J. Org. Chem.* **2004,** *69*, 3417.
35. SIRT1 (#SE239) and SIRT2 (#SE251) were bought from BIOMOL; SIRT1-2 Fluorimetric Drug Discovery Kit (#AK-555, #AK-556). The assays were performed using a modified protocol based on the Fluor de lys fluorescence protocol described by BIOMOL. Cbz-Lys(Ac)-AMC (synthesized in-house) was used as the substrate.
36. HPLC conditions: column OD-RH (150 x 4.6 mm, 5 mm, inj. vol. 5 µL, Daicel Chemical Industries); run time 60 min, flow rate 0.5 ml/min, 20% CH3CN/H2O to 80% CH3CN/H2O over 50 min then hold at 80% CH3CN/H2O for 10 min. Retention times for EX-527: 27.1 and 32.6 mins; compound 3b: 20.8 and 21.2 mins.
37. For instance, compound **3e** showed IC50 >10 µM for HDAC1,2,6,8 and no inhibition at 20 µM for HDAC 4,5,7.
38. Namalwa cells (50,000 cells/well) were plated in 96 well culture plates in a final volume of 100 µl. Test compounds were dissolved in DMSO and serial-dilutions were carried out with media mixture for final compound concentrations of 0.005 to 50 µM and DMSO concentration of 0.95%. Plates were incubated for 3 hours at 37 ºC under a 5% CO2 atmosphere. Cells were lysed for 30 min with 10 µl of 10X Lysis Buffer, and endogenous p53 protein present in 25 µl of cell lysate was captured by a p53 antibody (Santa-Cruz Cat# SC-126 DO-1) coated into the microwells of 96 well black plates (Nunc Maxisorp). A 25 µl of an acetylated-lysine382-p53 antibody (Cell Signaling Cat#2525) was added to detect the acetylated-lysine on p53 protein during an overnight incubation. After a washing step, 50 µl of an anti-mouse HRP antibody (Sigma #A-0545) was used to recognize the bound detection antibody; and after another washing step, 50 µl of Amplex Red solution was added to develop the reaction. Following a 15 min incubation period, fluorescence reading was performed on a fluorometer at excitation wavelength of 550 nm and emission of 610 nm. The magnitude of

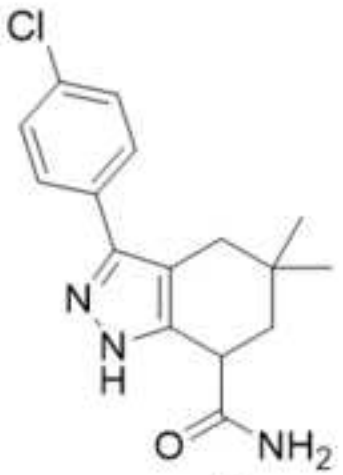
the fluorescence was proportional to the quantity of the acetylated p53 protein
39. Corbeil, C. R.; Englebienne, P.; Moitessier, N. *J. Chem. Inf. Model.* **2007,** *47*, 435.
40. Corbeil, C. R.; Moitessier, N. *J. Chem. Inf. Model.* **2009,** *49*, 997.
41. Using the displaceable water molecules mode in FITTED.





3b

IC_{50} (SIRT1) = 1.0 μM



13a

IC_{50} (SIRT1) = 0.8 μM