Brief Articles

Discovering Inhibitors of Human Sirtuin Type 2: Novel Structural Scaffolds

Anu J. Tervo,[†,‡] Tiina Suuronen,[§] Sergiy Kyrylenko,[§] Erkki Kuusisto,[§] Päivi H. Kiviranta,[†] Antero Salminen,[§,||] Jukka Leppänen,[†] and Antti Poso*[,†]

Departments of Pharmaceutical Chemistry and Neuroscience and Neurology, University of Kuopio, Post Office Box 1627, 70211 Kuopio, Finland, and Department of Neurology, Kuopio University Hospital, Post Office Box 1777, 70211 Kuopio, Finland

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A successful virtual screening experiment of novel SIRT2 inhibitors is described. Four out of 11 experimentally tested compounds showed in vitro inhibitory activity toward SIRT2 in a micromolar level, resulting in an experimental hit ratio of 36%. Two of these compounds inhibited SIRT2 with IC_{50} (μM) values of 51 and 91; moreover, one of the new inhibitors was comprised of an entirely new SIRT2-inhibiting structural scaffold.

Introduction

The silent information regulator Sir2 and other Sir2-like proteins (sirtuins)[1] belong to the Class III of histone deacetylases (HDACs).[2] The in vitro deacetylation reaction of the acetylated lysines of histones H3 and H4 catalyzed by Sir2 in yeast is dependent on the presence of the cofactor nicotinamide adenine dinucleotide (NAD^+),[3] and the products of the catalyzed deacetylation reaction are nicotinamide, a deacetylated histone protein and *O*-acetyl-ADP-ribose.[4,5] In addition, some of the Sir2 family members have been demonstrated to deacetylate nonhistone proteins, such as the tumor suppressor protein p53.[6]

Until recently, seven human Sir2 homologue proteins have been identified.[7,8] Type 2 from these human sirtuins, SIRT2, is predominantly a cytoplasmic protein except in the G2/M transition and in mitosis, where it is localized in association with chromatin.[9] It has been shown to deacetylate α-tubulin in vivo, to target histones with a preference for histone H4 Lys16 during mitosis,[9,10] and to control mitotic exit within cell cycles[11] in vitro.

Several reports describe the role of HDAC inhibitors as new anticancer agents, and a number of these inhibitors have already entered into clinical trials.[12−15] Human sirtuins could be potential therapeutic targets related to, for example, the treatment of some cancers[16,17] or the modification of physiological processes that may involve calorie restriction such as the aging process,[18] fat storage,[19,20] or Alzheimer's disease.[21] Moreover, it has been suggested that the sirtuin-dependent deacetylation cannot be compensated by the Class I−II HDACs, and that combining a Class I or II HDAC inhibitor with a sirtuin inhibitor could increase their overall chemotherapeutic efficacy.[22,23] Thus, there is an increasing interest in the development of sirtuin inhibitors,[24] and some small molecule inhibitors for sirtuins have already been described in the literature.[17,25−30] For SIRT2, so far discovered inhibitors include for example **1** (sirtinol),[27] **2** (A3),[27] **3** (CD04097),[28] **4** (JFD00244),[28] **5** (Cambinol),[17] and **6** (35)[30] (Figure 1). Despite these and other known inhibitors, more structurally diverse inhibitors of SIRT2 need to be discovered for improving the understanding of the biological function of SIRT2 and to discover its potential therapeutic indications.

Experimental Section

Based on our previous results[28] and examination for favorable interactions between the docked (Gold 2.0)[31] inhibitors **1**−**4** and the putative SIRT2 active site,[32] we targeted our interest to the side chains of Asp95 and Asp170 as potential hydrogen bond acceptors to the side chain of Gln167 as a potential hydrogen bond donor and to the side chains of Phe119 and Phe235 for hydrophobic contacts. On the basis of these observations, two receptor-based virtual screening queries were conducted, and the resulting compounds from virtual screen were required to fulfill the following criteria. The queries contained excluded volumes to outline the putative active site, a hydrophobic feature near Phe119 and Phe235, a donor atom near Ile169 and Asp170, and another donor near Asp95; in addition, the second query contained an acceptor atom near Gln167 as an alternative to the latter donor. Applying these interactions in virtual screening of Maybridge Screening Collection[33] and LeadQuest[34] databases (Unity 4.4 implemented in Sybyl 6.9), followed by a visual examination of the resulting compounds, we chose 11 compounds for in vitro testing. The efficiency of SIRT2 inhibitors was tested in vitro using human recombinant SIRT2 enzyme and the fluorescent *Fluor de Lys* SIRT2 substrate (KI179) from BIOMOL (Plymouth Meeting, U.S.A.). Recombinant human SIRT2 enzyme was expressed as a GST fusion protein in *E. coli* and purified as we have described recently.[28]

Results and Discussion

Four of 11 tested compounds showed SIRT2 inhibitory activity ≤200 μM (compounds **7**−**10**; Table 1 and Figure 2). This results in an experimental hit ratio of 36%, which demonstrates the success of the virtual screening experiment. The rest of the compounds yielded IC_{50} values of over 200 μM (data not shown) and were considered as inactives. Compounds **7** and **9** resulted in IC_{50} (μM) values of 91 and 51, respectively, being equipotent with the inhibitors **1**−**4**. Therefore, **7** and **9** can be considered as novel inhibitors of SIRT2.

The two novel SIRT2 inhibitors contain four hydrogen-bond donors. The number of donor atoms is higher than the average

* To whom correspondence should be addressed. Tel.: +358-17-162462. Fax: +358-17-162456. E-mail: antti.poso@uku.fi.
† Department of Pharmaceutical Chemistry, University of Kuopio.
‡ Current address: AstraZeneca R&D Mölndal, Pepparedsleden 1, SC2, 43183 Mölndal, Sweden.
§ Department of Neuroscience and Neurology, University of Kuopio.
|| Kuopio University Hospital.

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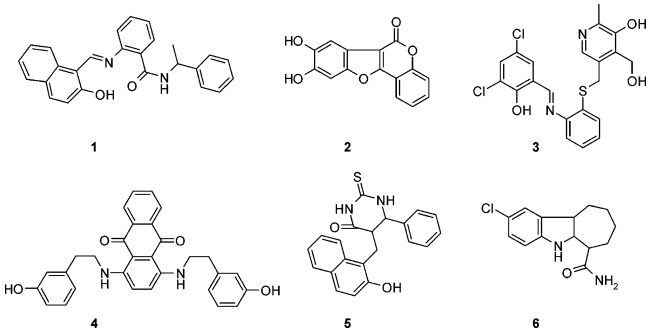

Figure 1. Known inhibitors of SIRT2.

Table 1. Inhibition of SIRT2 Activity by Compounds **1** and **3**−**10**

compd[a]	code	IC_{50} (μM)[d]
1	sirtinol	45[e]
3	CD 04097[b]	74[e]
4	JFD00244[b]	57[e]
5	cambinol	59[f]
6	35	2.8[g]
7	CD 03241[b]	91
8	TRIPOS 354328[c]	136
9	TRIPOS 360702[c]	51
10	TRIPOS 551502[c]	200

[a] The structures of the compounds are presented in Figures 1 and 2. [b] Compound code name in the Maybridge database. [c] Compound code name in the LeadQuest database. [d] Measured inhibitory activity expressed as the IC_{50} of SIRT2. Average values from the IC_{50} determinations performed in triplicate are presented. Standard deviations were ≤30%. [e] IC_{50} values were taken from our recent publication.[28] [f] IC_{50} values from Helweg et al.[17] [g] IC_{50} values from Napper et al.[30]

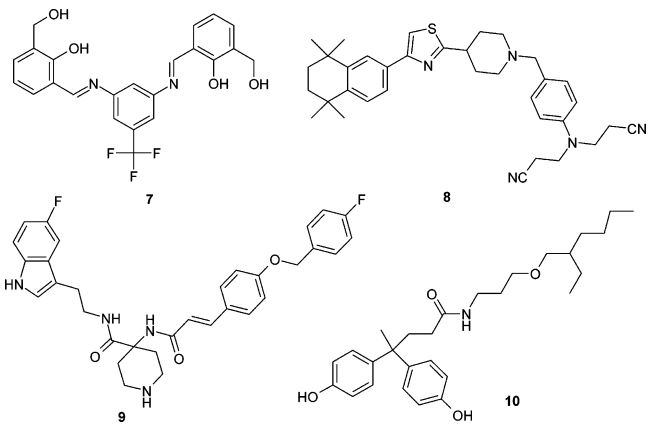

Figure 2. Structures of the four new compounds that showed SIRT2 inhibitory activity.

of two donors that are found in inactive compounds. The significance of hydrogen-bond donating property to the SIRT2 inhibitory activity was also suggested previously.[28] The number of hydrogen-bond acceptors could not be used to separate the active and inactive compounds; this was also the case for molecular weights and the scoring function values of the docked poses.

According to our docking results, the inhibitors **1**−**4**, **7**, and **9** interact with the side chain carboxyl oxygen of Asp95 (**2**, **4**, and **9**), with Gln167, mostly by forming a hydrogen bond to its backbone carboxyl oxygen (all except for **2**), with the side chain carboxyl group of Asn168 (**1** and **7**), and with the backbone NH group of Ile169 (**1** and **4**). Thus, the majority of the hydrogen-bonding interactions of these inhibitors takes place at the region Gln167-Asn168-Ile169-Asp170; therefore, on the basis of this study, it seems that this region is important for the inhibitor binding. This is also supported by the finding that the docked inactive compounds were almost completely lacking hydrogen bonds within this area. Most of the docked inhibitors also had favorable interactions with hydrophobic amino acids of the putative SIRT2 active site.

Interestingly, the inhibitor **9** is comprised of an entirely new SIRT2-inhibiting structural scaffold. A phenolic or a 2-hydroxynaphthaldehyde moiety has been previously suggested of being primarily responsible for the inhibitory activity of the known SIRT2 inhibitors.[27,28] These moieties might be important to the binding of compounds interacting with human sirtuins, because the activators of SIRT1 and most of the inhibitors of SIRT2 known so far contained a phenolic moiety in their structures.[24,28] However, the inhibitor **9** and the recent findings of Napper et al.,[30] who reported a series of indole derivatives as specific SIRT1 inhibitors with micromolar SIRT2 IC_{50} values, demonstrate that a phenolic or a 2-hydroxynaphthaldehyde moiety is not required for the SIRT2 inhibitory activity. These findings also result in a reformation of the previous suggestion for a SIRT2 inhibitor pharmacophore.[28] Instead, a common pharmacophore for these inhibitors could be comprised of a minimum of two hydrogen-bond donors and a hydrophobic moiety to match the hydrophobic nature of the putative SIRT2 active site.

Two representatives of the most commonly occurring docked conformation of the novel inhibitor **9** were examined in more detail within the putative SIRT2 active site (Figure 3). The docked orientations were mostly differing in respect to the 2-(5-fluoroindol-3-yl)-ethylamine moiety. According to the docking results, the NH group of the 5-fluoroindole moiety could form a hydrogen bond to the backbone carboxyl oxygen of Gln167 in the first of these docked orientations. Another hydrogen bond could occur from the NH group of the piperidine ring to the side chain carboxyl oxygen of Asp95. The 5-fluoroindole moiety was located near Ala186, His187, and Phe235, while the piperidine ring was located near Phe96, Arg97, Gln167, and

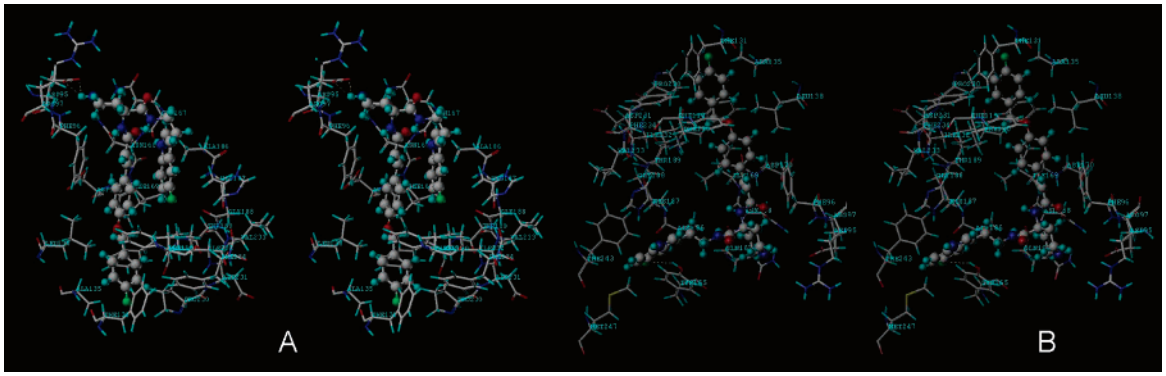

Figure 3. Structurally novel inhibitor **9** (ball-and-stick) as docked at the putative SIRT2 (capped stick) active site. A and B corresponds to two main docking poses. Potential hydrogen bonds are indicated using dashed line.

Asn168. The second docked orientation represented the most often occurring orientation among the docked poses of **9**. Here, the 5-fluoroindole moiety was located near His187, Phe243, and Met247, and a hydrogen bond could occur between the hydroxyl group of Tyr165 and the fluorine of this moiety. The piperidine ring was again located near Phe96, Arg97, Gln167, and Asn168; however, no hydrogen bonding was observed. In both docked orientations, the rest of the compound was pointing toward the hydrophobic pocket located near Phe119, Phe131, Ala135, Leu138, Ile169, Phe190, Pro230, and Phe234.

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Note Added after ASAP Publication. This manuscript was released ASAP on November 9, 2006, with errors in Table 1. The correct version was posted on November 27, 2006.

Supporting Information Available: Virtual screening procedure and the applied SIRT2 activity assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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