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Spiro heterocycles as potential inhibitors of SIRT1: Pd/C-mediated synthesis of novel *N*-indolylmethyl spiroindoline-3,2′-quinazolines

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ABSTRACT

Novel *N*-indolylmethyl substituted spiroindoline-3,2′-quinazolines were designed as potential inhibitiors of SIRT1. These compounds were synthesized in good yields by using Pd/C–Cu mediated coupling-cyclization strategy as a key step involving the reaction of 1-(prop-2-ynyl)-1′*H*-spiro[indoline-3,2′-quinazoline]-2,4′(3′*H*)-dione with 2-iodoanilides. Some of the compounds synthesized have shown encouraging inhibition of Sir 2 protein (a yeast homologue of mammalian SIRT1) in vitro and three of them showed dose dependent inhibition of Sir 2. The docking results suggested that the benzene ring of 1,2,3,4-tetrahydroquinazolin ring system of these molecules occupied the deep hydrophobic pocket of the protein and one of the NH along with the sulfonyl group participated in strong H-bonding interaction with the amino acid residues.

Indoles being the most abundant N-heterocycle in drugs, pharmaceuticals, natural products, and agrochemicals have attracted enormous attention over the years.[1] Indeed, the indole framework is considered as one of the most privileged structures in the area of medicinal chemistry and drug discovery. The spiro heterocycles on the other hand have gained particular attention because of their interesting pharmacological properties in addition to their natural occurrences. These include spiroindole based bioactive natural products, for example, horsfiline (**A**), spirotryprostatin A (**B**), coerulescine (**C**), etc. (Fig. 1).[2,3] Recently, due to their wide range of pharmacological properties[4–6] spiroquinazolines have attracted considerable interest in medicinal and pharmaceutical chemistry. Thus spiroindoline-3,2′-quinazolines possessing a *N*-indolylmethyl substituent appeared to be an interesting class of heterocycles and development of appropriate synthetic methodologies is desirable for accessing these novel compounds.

In view of traumatic side effects of existing anticancer drugs there is an urgent need for the development of more specific and relatively non toxic drugs to address the major health problem of cancer worldwide.[7a] Epigenetic therapeutics of cancer such as inhibitors of DNA methyltransferases and histone deacetylases (class I and class II) are already being used in combination with the standard cytotoxic agents.[7b] The Sirtuins (class III NAD-dependent deacetylases that catalyze NAD+ dependent removal of acetyl group to generate deacetylated proteins, nicotinamide, and *O*-acetyl-ADP-ribose) function in diverse biological processes such as transcriptional silencing, regulation of apoptosis by deacetylation of p53, fatty acid metabolism, cell cycle regulation, and aging.[8] The mammalian sirtuin family consists of seven members, for example, SIRT1–7 and among the seven human sirtuins, SIRT1 has been studied well which has several substrates such as p53, Ku70, NF-κB, forkhead proteins, etc.[9] Sirtuins are being considered as important targets for cancer therapeutics as they are up-regulated in many cancers. Inhibition of sirtuins allows re-expression of silenced tumor suppressor genes, leading to reduced growth of cancer cells. Several small molecule inhibitors of sirtuins, such as nicotinamide, sirtinol, splitomicin, cambinol, tenovins, and the indole derivative EX527[10] have been shown to induce cell death in cancer cells. However, no sirtuin inhibitors except EX527 (which is presently undergoing Phase 1a clinical trial for the treatment of Huntington's disease) have progressed into clinical trials as anticancer agents. While indole derivates, for example, EX527 have been explored as inhibitors of sirtuins earlier the sirtuin inhibiting properties of indoles containing spiro heterocycles has not been examined. Moreover, structural manipulations of a generic spiroindole **D** via **E** followed by incorporating some of the structural features of EX527 lead to spiro heterocycles **F** (Fig. 2). Indeed, the design and selection of 1,2,3,4-tetrahydroquinazolin ring as a part

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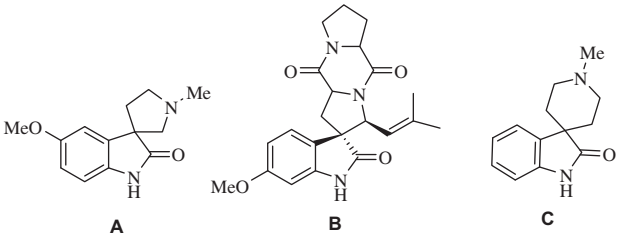
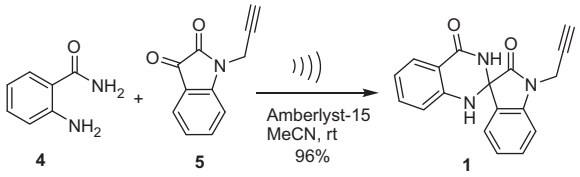


Figure 1. Examples of spiro heterocycle based bioactive natural products.



Scheme 2. Preparation of 1-(prop-2-ynyl)-1′*H*-spiro[indoline-3,2′-quinazoline]-2,4′(3′*H*)-dione (**1**).

of spiro heterocycles was supported by the docking studies (homology modeling) performed using a series of molecules based on **F** with human SIRT1 (hSIRT1) (see later for a discussion). This prompted us to synthesize and evaluate the SIRT1 inhibiting properties[11] of **F** and herein we report our preliminary results of this study, that is, *N*-indolylmethyl substituted spiroindoline-3,2′-quinazolines as potential inhibitors of SIRT1.

A large number of methods[1,12,13] have been reported for the construction of indole ring many of which are mediated by transition metal catalysts particularly palladium catalysts. The use of Pd/C–CuI–PPh_3 as a less expensive catalyst system for efficient synthesis of various heterocyclic structures[14] including indoles[15,16] has been explored earlier. The catalyst Pd/C is stable and easy to handle as well as separable from the product. Moreover, the catalyst can be recycled.[14] In view of simplicity, advantages and versatility of this methodology we decided to adopt a similar Pd/C based coupling-cyclization strategy as a key step for the synthesis of our target compounds **F** (or **3**) as shown in Scheme 1.

Thus, the reaction of 1-(prop-2-ynyl)-1′*H*-spiro[indoline-3,2′-quinazoline]-2,4′(3′*H*)-dione **1** with 2-iodoanilides (**2**) in the presence of 10% Pd/C, CuI, PPh_3 and Et_3N in EtOH at 70 °C afforded the corresponding *N*-indolylmethyl substituted spiroindoline-3,2′-quinazolines (**3**) via a tandem coupling-cyclization process in the same pot. The key starting material, that is, the terminal alkyne **1** required for our study, was prepared by reacting 2-aminobenzamide (**4**) with 1-(prop-2-ynyl)indoline-2,3-dione (**5**) under ultrasound irradiation at room temperature (Scheme 2). All the 2-iodoanilides (**2**) were prepared according to the procedure described in the literature.[15b,17] Initially, we chose to examine the coupling reaction of 1-(prop-2-ynyl)-1′*H*-spiro[indoline-3,2′-quinazoline]-2,4′(3′*H*)-dione **1** with *N*-(2-iodophenyl)methanesulfonamide (**2a**) in the presence of 10% Pd/C (0.026 equiv), PPh_3 (0.20 equiv), CuI (0.05 equiv), and triethylamine (3.0 equiv) in various solvents. The corresponding results are summarized in Table 1. The reaction was initially carried out in ethanol for 3 h when the desired product, that is, 1-((1-(methylsulfonyl)-1*H*-indol-2-yl)-methyl)-1′*H*-spiro[indoline-3,2′-quinazoline]-2,4′(3′*H*)-dione (**3a**) was isolated in 80% yield (Table 1, entry 1). The increase of reaction time did not improve the product yield further (Table 1, entry 2). The use of other solvents such as MeOH, MeCN, 1,4-dioxane and DMF was examined and found to be less effective in terms of product yields (Table 1, entries 3–6). Since best result was achieved by using EtOH as a solvent hence all other studies were carried out using EtOH.

Having established the optimum reaction conditions for the preparation of **3a** we then used this methodology for the preparation of our other target compounds related to **3a**. Thus, a variety of 2-iodoanilides (**2**) were employed under the reaction conditions presented in entry 1 of Table 1 and the results are summarized in Table 2.

As evident from Table 2 that the reaction proceeded well with other 2-iodoanilides (Scheme 1 and Table 2). Various groups such as Cl (**3d**, **3k**), F (**3b**, **3i**), Br (**3c**, **3j**), Me (**3f**, **3m**) and CF_3 (**3e**, **3l**) were tolerated. All the indole derivatives (**3a**–**n**) synthesized were characterized by spectral data.

All the synthesized compounds were tested for sirtuin inhibitory potential in vitro by using a yeast cell based reporter silencing assay as a model system for primary screening. Compounds were tested at the concentration of 50 μM for their ability to inhibit yeast sirtuin family NAD-dependent histone deacetylase (HDAC) Sir 2 protein (a yeast homologue of mammalian SIRT1). Splitomi-

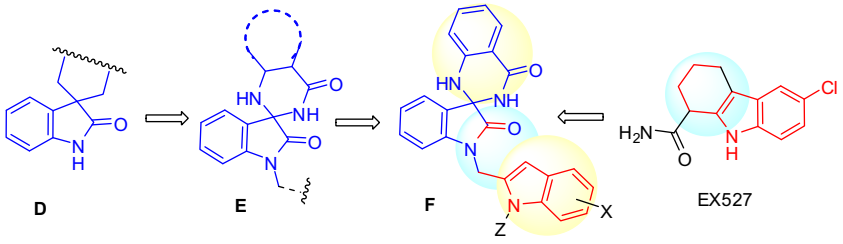
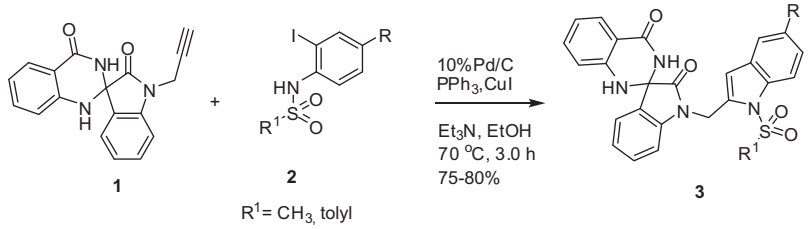
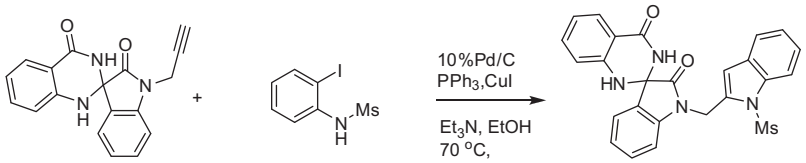


Figure 2. Design of novel spiro heterocycles **F** from coerulescin (**C**) and the known inhibitor EX527.



Scheme 1. Pd/C-mediated synthesis of *N*-indolylmethyl substituted spiroindoline-3,2′-quinazolines (**3**).

Table 1
Effect of solvents on Pd/C-mediated reaction of 1-(prop-2-ynyl)-1′*H*-spiro[indoline-3,2′-quinazoline]-2,4′(3′*H*)-dione (**1**) with *N*-(2-iodophenyl)methanesulfonamide[a] (**2a**)

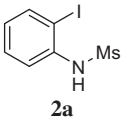
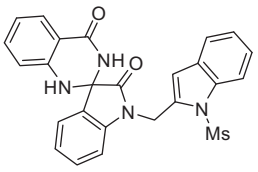
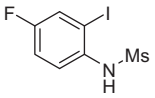
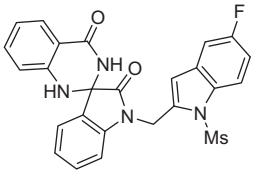
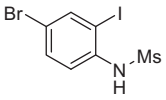
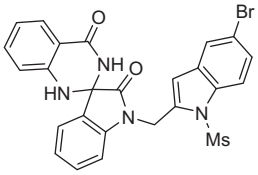
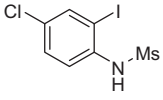
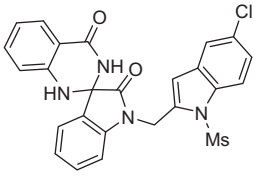
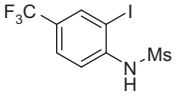
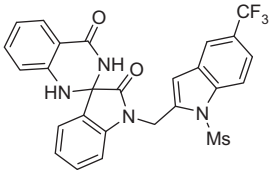


1 **2a** **3a**

Entry	Solvent	Time (h)	Yield[b] (%)
1	EtOH	3	80
2	EtOH	6	80
3	MeOH	3	75
4	MeCN	10	60
5	1,4-Dioxane	3	65
6	DMF	3	60

[a] All the reactions were carried out by using **1** (1.0 equiv), **2a** (1.5 equiv), 10% Pd/C (0.026 equiv), PPh_3 (0.20 equiv), CuI (0.05 equiv), and Et_3N (3 equiv) in EtOH at 70 °C under a nitrogen atmosphere.
[b] Isolated yields.

Table 2
Pd/C-mediated synthesis of *N*-indolylmethyl substituted spiroindoline-3,2′-quinazolines (**3**) (Scheme 1)[a]

Entry	2-Iodoanilides (**2**)	Product (**3**)[b]	Yield[c] (%)
1	 **2a**	 **3a**	80
2	 **2b**	 **3b**	78
3	 **2c**	 **3c**	75
4	 **2d**	 **3d**	80
5	 **2e**	 **3e**	75

(*continued on next page*)

Table 2 (*continued*)

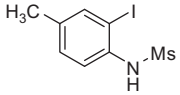
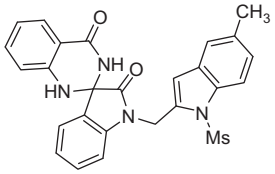
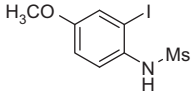
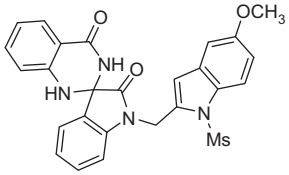
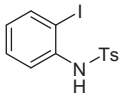
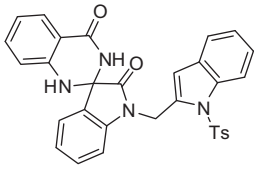
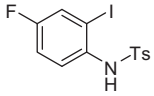
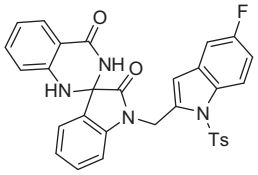
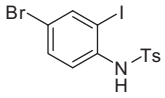
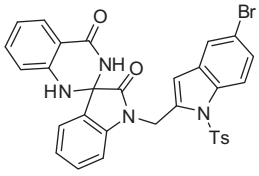
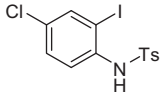
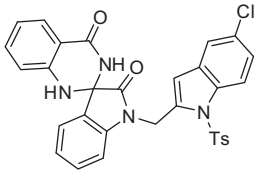
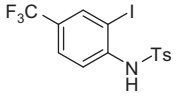
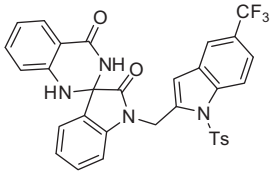
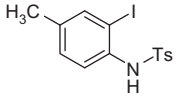
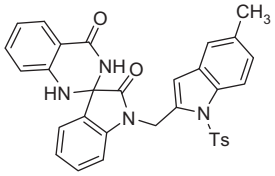
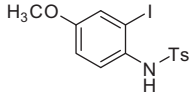
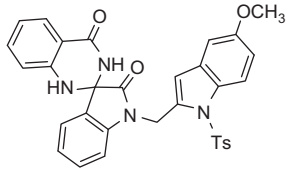
Entry	2-Iodoanilides (**2**)	Product (**3**)[b]	Yield[c] (%)
6	 **2f**	 **3f**	80
7	 **2g**	 **3g**	75
8	 **2h**	 **3h**	78
9	 **2i**	 **3i**	75
10	 **2j**	 **3j**	80
11	 **2k**	 **3k**	75
12	 **2l**	 **3l**	80
13	 **2m**	 **3m**	75

Table 2 (*continued*)

Entry	2-Iodoanilides (**2**)	Product (**3**)[b]	Yield[c] (%)
14	 **2n**	 **3n**	78

[a] All the reactions were carried out by using **1** (1.0 equiv), **2** (1.2 equiv), 10% Pd/C (0.026 equiv), PPh_3 (0.20 equiv), CuI (0.05 equiv), and Et_3N (3 equiv) in EtOH at 70 °C under a nitrogen atmosphere.
[b] Identified by ^1H NMR, IR, HPLC and MS.
[c] Isolated yields.

Table 3
Yeast based in vitro bioassay of *N*-indolylmethyl substituted spiroindoline-3,2′-quinazolines (**3**)

Entry	Compounds (**3**)	% Inhibition @ 50 μM[a]
1	**3a**	13.9
2	**3b**	30.3
3	**3c**	38.5
4	**3d**	30.9
5	**3e**	39.6
6	**3f**	40.9
7	**3g**	43.5
8	**3h**	36.5
9	**3i**	15.2
10	**3j**	16.4
11	**3k**	40.9
12	**3l**	38.4
13	**3m**	1.2
14	**3n**	42.4

[a] Data represent the mean values of three independent determinations. Splitomicin was used as a reference compound.[18a]

cin,[18a] a known inhibitor of sirtuin, was used as a reference compound in this assay. Various *N*-indolylmethyl substituted spiroindoline-3,2′-quinazolines (**3**) were tested for their ability to inhibit Sir 2 protein by estimating inhibition of growth of yeast strain containing URA3 gene at telomeric locus, in presence of 5-fluoroorotic acid (5-FOA).[18b] In this assay a yeast strain (TEL::URA3 strain (MATα ura3-52 lys2-801 ade2-101 trpΔ63 his3Δ200 leu3Δ200 leu2-Δ1 TEL adh4::URA) was used in which, a reporter gene

Table 4
Dose dependent % inhibition shown by compounds **3g**, **3k** and **3n**

Concentration (μM)	% Inhibition[a]		
	3g	**3k**	**3n**
1	9	18	12
10	20	30	25
30	29	37	32
50	39	41	41
100	67	56	61

[a] Data represent the mean values of three independent determinations.

URA3 was inserted in the silenced telomeric region where it is silenced by yeast Sir 2 protein. Inhibition of Sir 2 protein by an inhibitor would allow the URA3 gene to be expressed thereby resulting in death of the yeast cell in presence of 5-FOA through the formation of toxic 5-fluorouracil. The toxicity of test compounds can also be tested by using this assay. For example, the cells when grown in the absence of 5-FOA should grow if the compound is not toxic whereas in case of toxic compound yeast cells would die. Nevertheless, the results of our studies using the yeast based in vitro bioassay are summarized in Table 3. It is evident that a substituent at C-5 position of the *N*-mesyl indole ring of **3** played a key role in Sir 2 inhibition. For example increased activities were observed when this position was occupied by F, Br, Cl, CF_3, Me or OMe (Table 3, entries 1 vs 2–7). A slightly different trend was observed in case of *N*-tosyl derivatives (Table 3, entries 8–14) where a compound without C-5 substitution on the indole ring

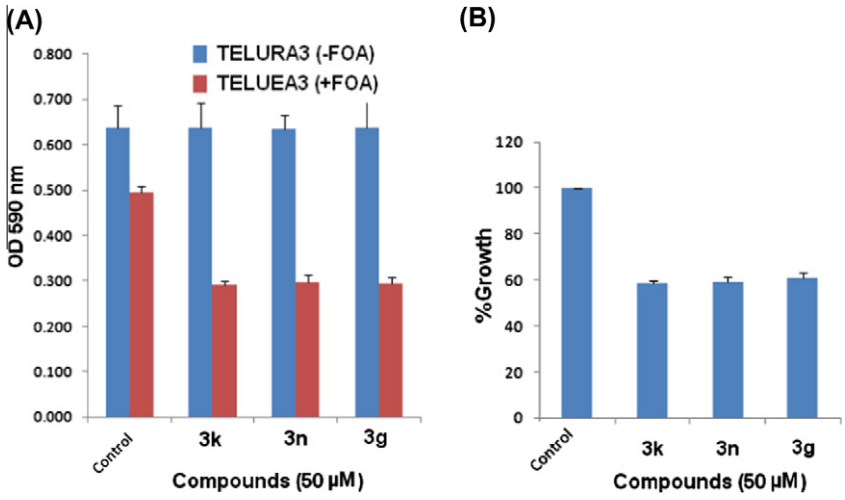


Figure 3. Inhibition of Sir 2 protein mediated transcriptional silencing at the telomeric locus in yeast by **3k**, **3n** and **3g**. (A) The growth inhibition of yeast in presence of **3k**, **3n** and **3g** which is due to inhibition of HDAC activity of Sir 2 protein. (B) Representative % growth inhibitory activity of the compound **3k**, **3n** and **3g**.

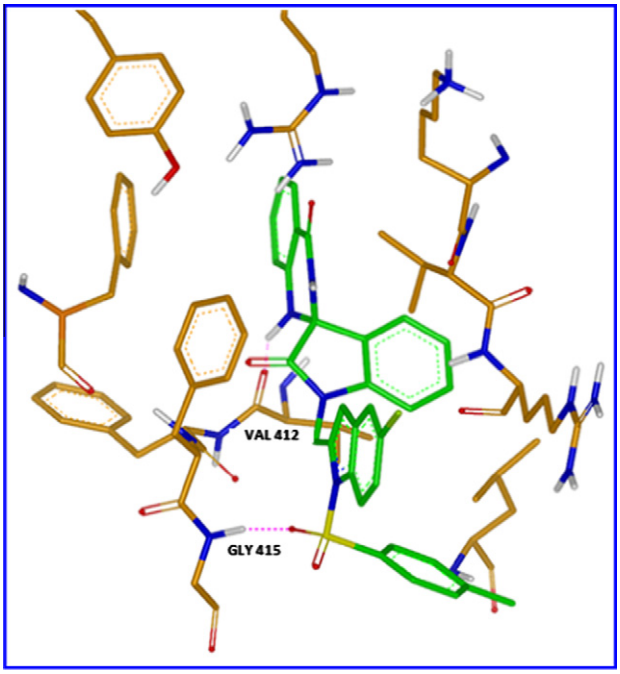


Figure 4. Binding interaction (H-bonding) and docked pose of **3k** at the hSIRT-1 catalytic site.

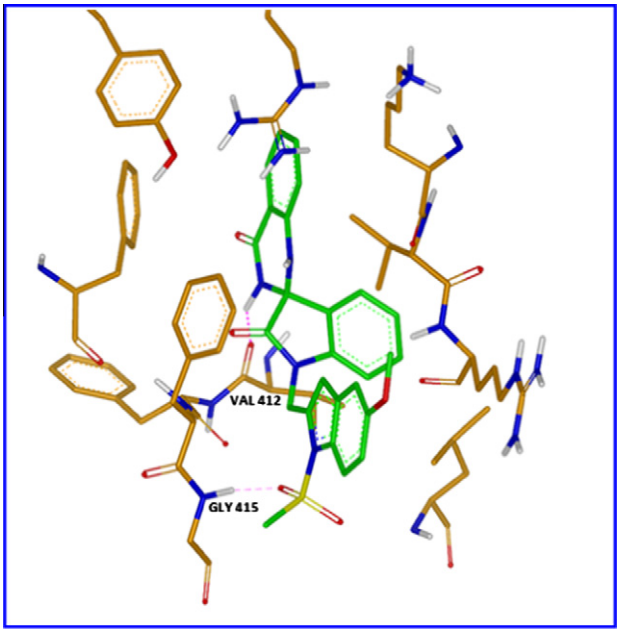


Figure 6. Binding interaction (H-bonding) and docked pose of **3g** at the hSIRT-1 catalytic site.

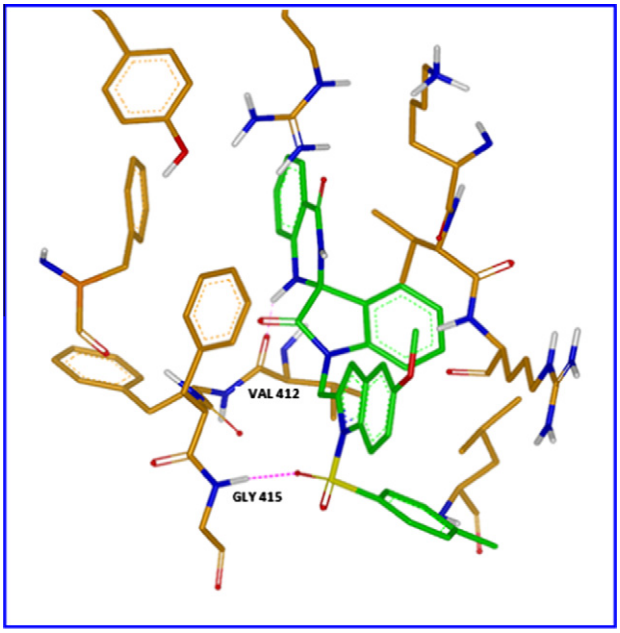


Figure 5. Binding interaction (H-bonding) and docked pose of **3n** at the hSIRT-1 catalytic site.

showed significant inhibition, for example, **3h**. Moreover, compound having F or Br or Me at C-5 was found to be inferior to **3h**, for example, **3i**, **3j** and **3m**. It is evident from Table 3 that among all the compounds tested **3g**, **3k** and **3n** showed significant inhibition in the presence of 5-FOA (Fig. 3). None of these compounds showed significant toxic effect as can be seen from yeast growth in the absence of 5-FOA (Fig. 3). A dose response study was performed using these three compounds the results of which are summarized in Table 4.

In order to understand the preferred binding orientation of **3g**, **3k** and **3n** at the catalytic site of hSIRT1 (human SIRT1), the docking studies were performed, using FRED v3.0 implemented from OpenEye Scientific Software (for docking studies with Sir 2, see Supplementary data). The multi-conformer database of the inhibitors along with known inhibitor splitomicin[19] was generated by OMEGA v1.7.7.[20] The complexes formed by inhibitors with hSIRT-1 catalytic pocket are presented in Figures 4–6. The docking scores (Chemgauss4 score) summarized in Table 5 indicate that these molecules bind well with the hSIRT1 protein.[21] The study suggested that the benzene ring of 1,2,3,4-tetrahydroquinazolin ring system of these molecules occupied the deep hydrophobic pocket of the protein. The hydrogen of N1 atom of 1,2,3,4-tetrahydroquinazolin ring and sulfonyl group, in all three molecules participated in strong H-bonding interaction with oxygen of the backbone carbonyl group of VAL 412 and nitrogen of the backbone amino group of GLY 415, respectively (Fig. 7). These backbone H-bond interactions could be an important factor for stabilization of these inhibitors in the catalytic pocket of hSIRT1.

In conclusion, a series of novel *N*-indolylmethyl substituted spiroindoline-3,2′-quinazolines were designed as potential inhibitors of SIRT1. These compounds were synthesized in good yields by using Pd/C–Cu mediated coupling-cyclization strategy as a key step involving the reaction of 1-(prop-2-ynyl)-1′*H*-spiro[indoline-3,2′-quinazoline]-2,4′(3′*H*)-dione with 2-iodoanilides. Some of the compounds synthesized have shown encouraging inhibition of Sir 2 protein (a yeast homologue of mammalian SIRT1) when tested using yeast based in vitro bioassay. Three of them showed dose dependent inhibition of Sir 2. The docking results suggested that the benzene ring of 1,2,3,4-tetrahydroquinazolin ring system of these molecules occupied the deep hydrophobic pocket of the protein and one of the NH along with the sulfonyl group participated in strong H-bonding interaction with the amino acid residues. These interactions contributed towards the stabilization of these inhibitors in the catalytic pocket of hSIRT1. Overall, the synthetic strategy described here could be useful in constructing library of small molecules based on *N*-indolylmethyl substituted spiroindoline-3,2′-quinazoline framework. Additionally, the spiro heterocyclic framework presented here could be an attractive template for the identification of novel and potent inhibitors of SIRT1.

Table 5
Docking scores obtained after docking compounds **3k**, **3n** and **3g** into SIRT1 protein

Molecules	Dock score[a]	Steric	Protein desolvation	Ligand desolvation H-bond	Clash	Ligand desolvation	Hydrogen bond
Splitomicin	−9.9	−13.9	−3.9	−0.1	0.2	0.3	−0.3
3k	−7.6	−17.4	7.5	−0.9	0.9	3.6	−1.4
3n	−7.9	−17.0	6.0	−0.8	0.8	4.1	−1.1
3g	−7.3	−17.6	7.76	−0.8	0.9	3.8	−1.5

[a] FRED Chemgauss4 score.

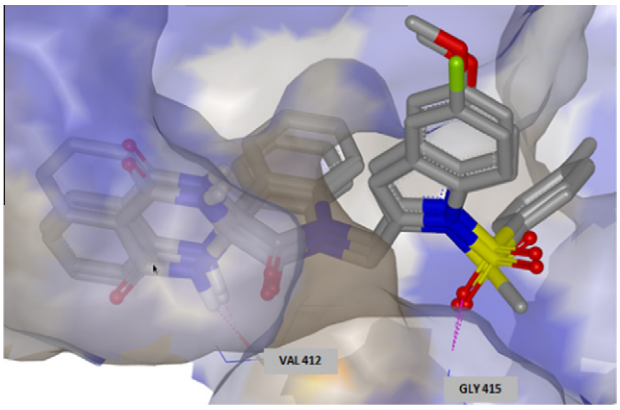


Figure 7. Binding mode and orientation of **3k**, **3n** and **3g** in hSIRT-1 catalytic pocket. Surface area colored in grey represents the hydrophobic property and blue the hydrophilic property.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.12.089.

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21. It is worthy to mention that while docking studies indicated similar binding orientation of **3g**, **3k** and **3n** at the catalytic site of hSIRT1 (which also correlates the observed in vitro results of these molecules) this observation however was specific for these molecules only and perhaps do not account the general binding mode of *N*-tosyl or *N*-mesyl derivatives with hSIRT1 and the observed trend of their in vitro inhibitions of yeast Sir 2.