Bioorganic & Medicinal Chemistry Letters 22 (2012) 3256–3260

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel acridinedione derivatives: Design, synthesis, SIRT1 enzyme and tumor cell growth inhibition studies

Mallika Alvala [a], Shubhmita Bhatnagar [a], Alvala Ravi [b], Variam Ullas Jeankumar [a], Thimmappa H Manjashetty [a], Perumal Yogeeswari [a], Dharmarajan Sriram [a,*]

[a] Drug Discovery Research Laboratory, Department of Pharmacy, Birla Institute of Technology and Science, Pilani-Hyderabad Campus, Hyderabad 500 078, India
[b] G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad 500 028, India

ARTICLE INFO

Article history:
Received 9 January 2012
Revised 27 February 2012
Accepted 7 March 2012
Available online 13 March 2012

Keywords:
Antiproliferative activity
Acridinedione derivatives
hSIRT1
MDA-MB231

ABSTRACT

A new scaffold *N*-(9-(*ortho*/*meta*/*para*-(benzyloxy)phenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl) isonicotinamide (**H1-3**) was discovered as a hSIRT1 inhibitor through virtual screening of *in-house* database. Based on these hits, a library of compounds were designed, synthesized and tested for in vitro hSIRT1 activity. The most potent compound **4d** in the series showed a significant inhibition of SIRT1 activity. Further antitumor studies of compound **4d**, showed a dose dependent increase in acetylation of p53K382 and decrease in SIRT1 with an IC_{50} of 0.25 μM in MDA-MB231 breast cancer cell lines. Individual 3D-QSAR analysis using Schrödinger showed distribution of hydrophobic and non polar positive co-efficient at *ortho* position essential for bioactivity based on **4d**.

SIRT1 also known as NAD-dependent deacetylase sirtuin-1, the closest mammalian homolog of yeast Sir2, belongs to the family of the class III histone deacetylase. The complicated interaction loops established between human sirtuins and various critical cellular pathways suggest them to be possible drug targets.[1–5] Several lines of evidence have indicated that SIRT1 deacetylates not only the lysine residues of histones, preferentially H3 and H4[6] but also non histone proteins. It has been found to be up-regulated in many tumor types and are able to deacetylate various substrates including p53,[7–9] BCL6,[10] HIV Tat,[11] PGC-1a,[12,13] AceCS1,[14] Ku70, Smad7, and NF-kB[15] and Rb[16] which are associated with various disease conditions such as cancer and HIV.[17] Therefore, SIRT1 inhibitors are of interest as potential therapeutic agents.[4] Several inhibitors of SIRT1 have been reported,[18] including nicotinamide,[18] EX-527 analogues,[19] splitomicin analogues[20–22] sirtinol analogues,[23] cambinol,[10] 2-anilinobenzamides,[24] aristoforin[25]and various other compounds reported[26–29] (Fig. 1). The first synthetic inhibitor that was discovered is sirtinol.[30] In the present study we describe the synthesis and structural characterization of a series of acridinedione derivatives as well as their hSIRT1 inhibitory activity supported with in silico, biochemical and in vitro cell-proliferation assay.

Since the crystal structure of human SIRT1 is not available, the three dimensional model of catalytic domain of the hSIRT1 (Q96EB6; 244–498 AA) was developed by comparative modeling using PRIME homology modeling program (Schrödinger L.L.C., USA) as described previously[31] and the fitness of the model was checked by PROCHECK program.[32] This model has 83.7% residues in most favored regions and ProSA-Web *Z* score of −6.38 similar to the template structure scores. Binding site pocket was identified by using SITEMAP (Schrödinger L.L.C., USA). With an aim of identifying novel hSIRT1 inhibitors, virtual screening of *in-house* database of 2500 molecules was carried out by using GLIDE (Schrödinger L.L.C., USA), GOLD and AUTODOCK 4.0 software's against catalytic core of hSIRT1. Based on docking score, binding energy and fitness values compounds H1, H2, and H3 were selected for (Fig. 2) in vitro enzymatic assay at 50 μM concentration along with reference compound suramin. Binding energy, docking score, fitness and percentage inhibition values are presented in Table 1.

Further to explore the structure–activity relationship of hit compounds (Fig. 2) as SIRT1 inhibitors, different derivatives were designed by computational and medicinal chemistry approaches. Docking studies for the designed compounds were performed by using three different softwares (Schrödinger, GOLD, and Autodock 4.0). Docking score, fitness values and binding affinity values of all the designed molecules along with reference compound suramin are shown in Table 2. The designed compounds having better scores than the initial hit compounds (H1-3), were derivatized by using a one pot synthetic protocol previously reported by our group.[33]

The target molecules (**4a–r**) were synthesized via a microwave assisted one-pot three component reaction (Scheme 1) of either *ortho*, *meta* or *para* substituted benzyloxy benzaldehydes (**1a–c**),

* Corresponding author. Tel.: +91 40 66303506; fax: +91 40 66303998.
E-mail address: drdsriram@yahoo.com (D. Sriram).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.03.030

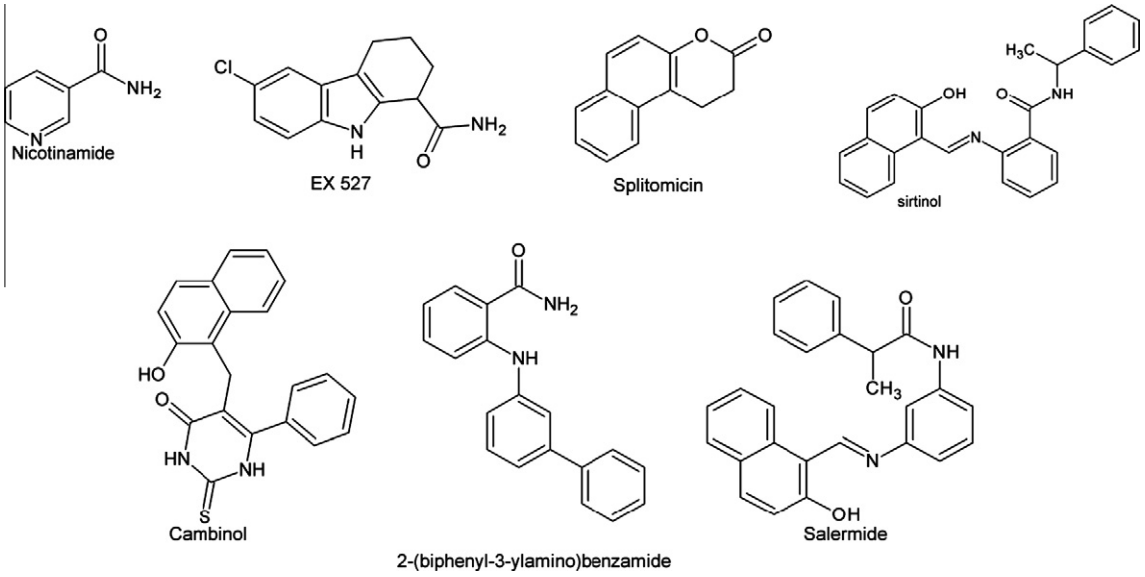


Figure 1. Known sirt1 inhibitors.

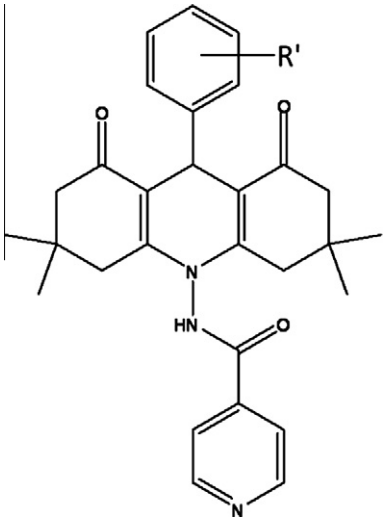


Figure 2. In-house database hit compound.

5,5-dimethylcyclohexane-1,3-dione (**2**), and corresponding phenyl hydrazine's or aryl/heteroaryl acid hydrazides (**3a–f**) in the ratio 1:2:1 in dry DMF media with catalytic amount of HCl. The use of microwave condition's in the present protocol not only improved the yield, but also reduced the reaction time drastically to 5–8 min compared to conventional heating which took about 3–8 h. Both analytical and spectral data (^{1}H NMR, ^{13}C NMR, and mass spectra) of all the synthesized compounds were in full agreement with the proposed structures.[34]

To explore the scope and limitations of these conditions a series of the six simplified derivatives, (**4a**–**r**) (Table 2) were prepared by introduction of either unsubstituted phenyl/benzoyl derivatives or with various electron withdrawing/donating groups like 2,4-dinitrophenyl or 4-nitrobenzoyl or 2,4-dichlorobenzoyl or 2,6-dimethoxynicotionoyl group at C_9 (R position) and also with either *ortho, meta* or *para* benzyloxy substituent on C_9 phenyl ring of aminoacridine dione [3,4,6,7-tetrahydro-3,3,6,6-tetramethyl-10-amino-9-phenyl acridine—1,8(2*H*,5*H*,9*H*,10*H*)—dione]. As apparent from the Table 2, the compounds were found to react well to give the corresponding amino acridinedione in excellent yields (64–84%).

The results of virtual screening were validated by *invitro* screening of the best hits (H1-3) against hSIRT1 activity. Among them H1 showed 72.63% inhibition at 50 μM and H2 and H3 were not significantly active. Based on these result we further generated a series of compounds and tested against hSIRT1 activity at 50 μM using the Fluor-de-Lys peptide substrate from Cayman chemicals (USA). Compounds showing more than 50% inhibition were selected for IC_{50} calculation. Compound **4a** and **4d** were found to be the most potent inhibitors compared to reference compound suramin with an IC_{50} of 11 ± 0.02 μM, and 10 ± 0.08 μM, respectively. Others like **4b, 4c, 4f, 4i, 4l,** and **4n** were found to be better inhibitors than suramin and the remaining compounds showed no inhibition. The percentage inhibition and IC_{50} values are presented in Table 2. The higher inhibitory activity of compounds **4a** and **4d** might be attributed to the presence of benzyloxy phenyl substitution at *ortho* position and the presence of phenyl and 2,4 dinitrophenyl groups. Binding pose of most active compound **4d** (Fig. 3A and B) clearly demonstrated the orientation of *ortho* benzyloxy group in the hydrophobic groove of the protein (red-hydrophobic; blue-hydrophilic).

It was observed that substituent at C_9 (–R position) of aminoacridine dione dictates the bioactivity, wherein introduction of bulky substituents like benzoyl or 4-nitrobenzoyl or 2,4-dichlorobenzoyl or 2,6-dimethoxynicotionoyl or shift of benzyloxy substituent (at R' position)on phenyl ring of aminoacridine dione to *meta* position showed either reduced or no inhibition (**4b, 4e, 4h, 4k, 4n, 4q**). However shift of benzyloxyphenyl substitution to *para* position resulted in moderate inhibition of enzyme in analogues **4c, 4f, 4i,** and **4r**.

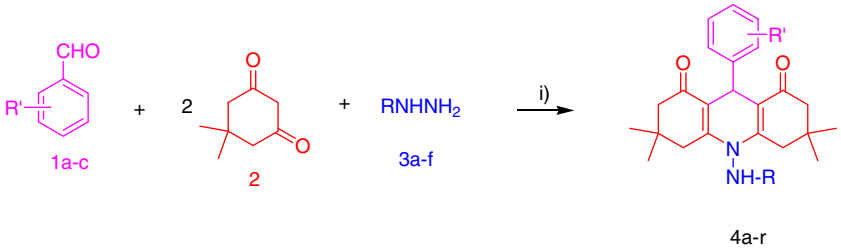
Over expression of SIRT1 is observed in many cancers and inhibition of SIRT1 by small molecules demonstrated inhibition of cancer cell proliferation.[35–37] To investigate the effectiveness of these SIRT1 inhibitors as anticancer agents, compounds **4a, 4b, 4c, 4d, 4f, 4i** and **4r** were evaluated for their ability to inhibit growth of tumor cells at 10 μM compared with suramin as standard SIRT1 inhibitor and doxorubicin as a standard anticancer drug. Human chronic myeloid leukemia (K562) cells and metastatic breast cancer (MDA-MB231) cells were used as tumor cells and HEK 293 as control cells. Percentage inhibition of **4a, 4b, 4c, 4d, 4f, 4i** and **4r** at 10 μM can be found in Figure 4. As shown in Table 2, all compounds showed better growth inhibition except **4a** in MDA-MB231 cells compared to K562 cells. There is no significant growth

Table 1
Docking scores, % inhibition of hsirt1 and cell growth inhibition at 50 μM concentration of hit compounds

	R′	Autodock score (Kcal/mol)	Gold Fitness	GLIDE Score	% inhibition of hSIRT1 at 50 μM	MDA-MB231 (IC_{50}μM)	K562 (IC_{50} μM)
H1	2-Benzyloxy	−6.79	52.50	−4.92	72.63343	5	10
H2	3-Benzyloxy	−7.46	57.84	−3.41	53.67	>50	10
H3	4-Benzyloxy	−7.68	55.80	−3.76	No inhibition @50 μM	>50	>50
Suramin		−4.67	45.7	−3.33	82.28	31.19	18.49

Table 2
Docking scores, physical constants and in vitro inhibition studies. (— indicates not determined)

No	R	R′	Yield (%)	% inhibition of hSIRT1 at 50 μM	Glide Docking Score	Gold fitness	Auto dock	SIRT1_IC_{50} (μM)	MDA MB231 IC_{50} (μM)
4a	Phenyl	2-Benzyloxy	84	82.1	−3.53	45.1	−8.85	11.08 ± 0.02	50
4b	Phenyl	3-Benzyloxy	75	51.4	−6.09	51.1	−8.95	40.06 ± 0.72	0.99
4c	Phenyl	4-Benzyloxy	64	55.2	−3.36	48.0	8.54	43.14 ± 0.1	1.38
4d	2,4-Dinitrophenyl	2-Benzyloxy	85	83.2	−4.28	57.6	−10.84	10.13 ± 0.08	0.25
4e	2,4-Dinitrophenyl	3-Benzyloxy	65	33.2	—	—	—	—	—
4f	2,4-Dinitrophenyl	4-Benzyloxy	75	54.8	−5.19	55.4	−8.52	43.29 ± 0.49	6.63
4g	Benzoyl	2-Benzyloxy	68	39.7	—	—	—	—	—
4h	Benzoyl	3-Benzyloxy	75	21.5	—	—	—	—	—
4i	Benzoyl	4-Benzyloxy	80	52.0	−5.90	51.9	−7.84	45.97 ± 0.71	3.16
4j	4-Nitrobenzoyl	2-Benzyloxy	77	39.0	—	—	—	—	—
4k	4-Nitrobenzoyl	3-Benzyloxy	73	33.6	—	—	—	—	—
4l	4-Nitrobenzoyl	4-Benzyloxy	65	35.6	—	—	—	—	—
4m	2,4-Dichlorobenzoyl	2-Benzyloxy	71	−19.7	—	—	—	—	—
4n	2,4-Dichlorobenzoyl	3-Benzyloxy	70	4.7	—	—	—	—	—
4o	2,4-Dichlorobenzoyl	4-Benzyloxy	69	3.6	—	—	—	—	—
4p	2,6-Dimethoxynicotionoyl	2-Benzyloxy	69	35.2	—	—	—	—	—
4q	2,6-Dimethoxynicotionoyl	3-Benzyloxy	79	35.6	—	—	—	—	—
4r	2,6-Dimethoxynicotionoyl	4-Benzyloxy	75	55.1	−3.08	42.8	−9.23	25.018 ± 0.02	0.31
	Suramin	—	—	53.9	−3.33	45.7	−4.67	2.8	
	Doxorubicin	—	—	—	—	—	—	—	10



Scheme 1. Synthesis of compounds. Reagents and conditions: (i) HCl, Dry DMF, MWI, 5–8 min.

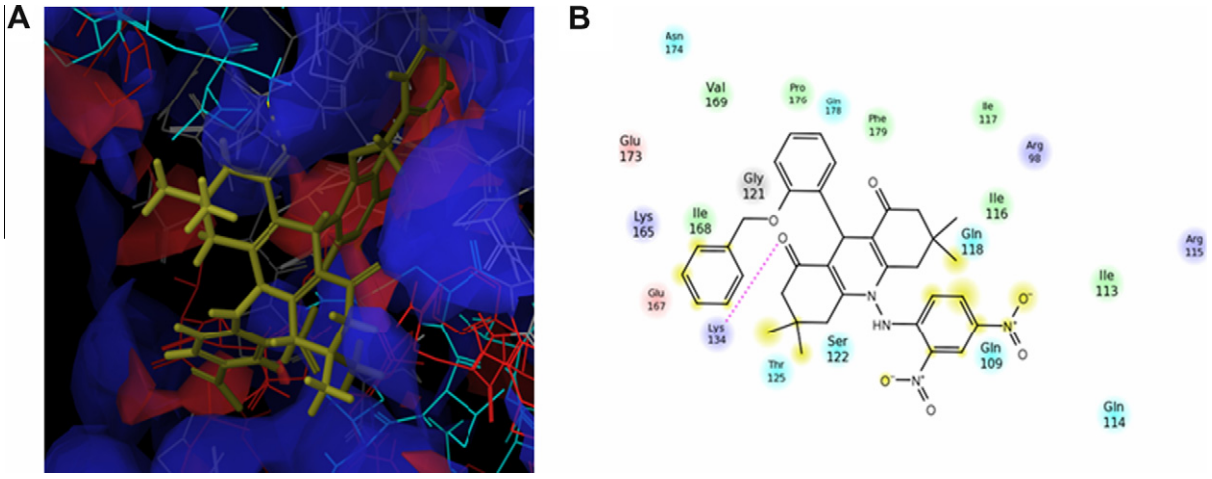


Figure 3. (A) Docking pose of most active compound **4d**. (B) Ligand interaction diagram showing important amino acids with in $5A^0$. Dotted line represents hydrogen bond to the side chain. Blue-charged positive, green-Hydrophobic group, Yellow-Solvent exposure, Red-acidic group, purple-basic, gray-other groups.

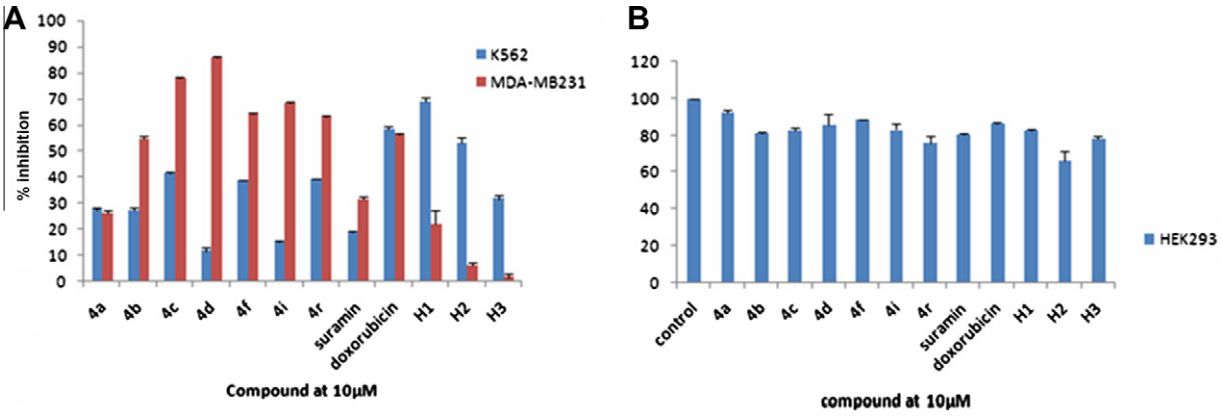


Figure 4. (A) Graph showing % inhibition on K562, MDA-MB231 cells. (B) Graph showing % growth on HEK 293 cells.

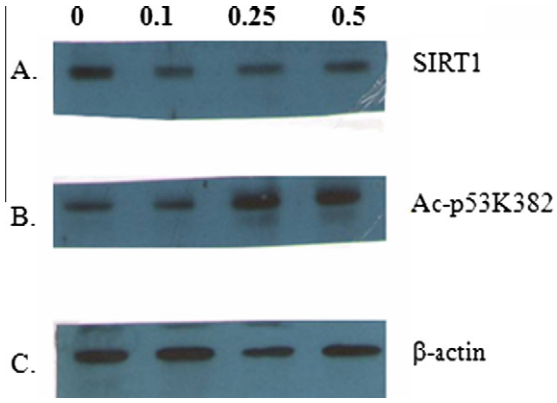


Figure 5. Western blot analysis of MDA-MB231 cells treated with compound **4d** at 0 μM, 0.1, 0.25 and 0.5 μM concentrations. (A) A representative Western blot showing the dose dependent decrease of expression levels of SIRT1. (B) Inhibition of SIRT1 by compound **4d** resulted in increased Acp53levels dose dependently. (C) β-actin was used as control.

Table 3
The QSAR results table shows the statistics of the fit for the training set and test set

	Activity(μM)	Predicted activity
4a	4.955	4.78347
4b	4.398	4.5601
4c	4.365	4.54989
4d	4.994	4.73862
4e	4.301	4.3524
4f	4.364	4.313
4g	4.301	4.3354
4h	4.301	4.289
4i	4.338	4.22116
4j	4.301	4.35061
4k	4.301	4.36279
4l	4.301	4.26662
4m	4.301	4.28422
4n	4.355	4.30994
4o	4.301	4.2141
4p	4.301	4.56794
4q	4.301	4.42566
4r	4.602	4.46849

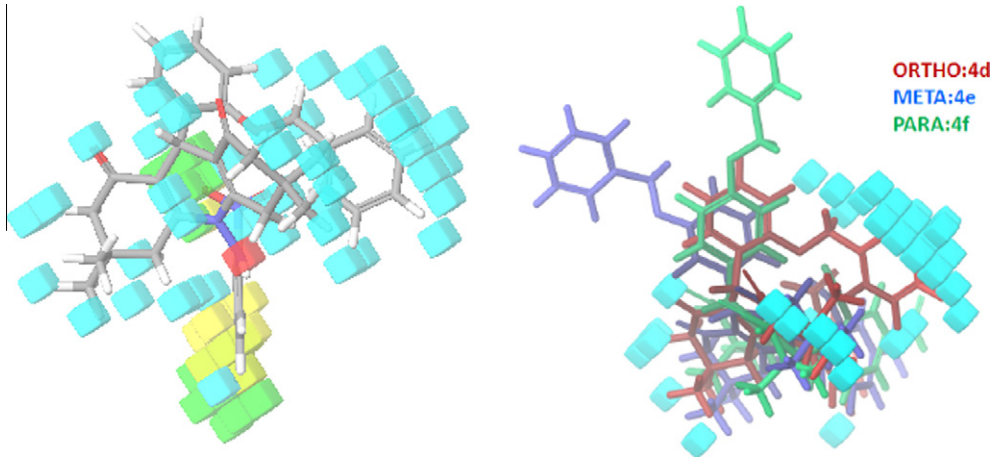


Figure 6. (A) QSAR visualization of most active compound **4d**. Cubes at that position shows positive threshold effect. Red-Hydrogen bond donor; Cyan-Hydrophobic groups; Green-Negative ionic; Yellow-Positive ionic; Pink-electron withdrawing groups. (B) QSAR of most active compound **4d** in which benzyloxy is present at *ortho, meta and para* positions (**4d, 4e, 4f**).

inhibition on HEK 293 cells indicating the specificity of these compounds towards cancer cells. Further, IC_{50} values were calculated on MDA-MB231 cells (Table 2). The compound **4d** emerged as the most promising inhibitor in MDA-MB231 cells with an IC_{50} of 250 nM. These results suggested that acridinedione derivatives bearing the benzyloxy substitution at *ortho* position are potential antitumor agents.

To investigate further the distinct effects of the compound **4d** as SIRT inhibitor, on cell fate, we performed Western blot analysis on MDA-MB231 cells treated with compound **4d** at different concentra-

tions (0, 0.1, 0.25 and 0.5 μM) for 24 h, to study their effects on the levels of SIRT1 and acetylation status of the SIRT1 target p53K382. Interestingly compound **4d** has shown dose dependent decrease in SIRT1 (Fig. 5A) and increase in acetylation of p53 at lysine 382 (Fig. 5B). These results are in accordance with the previous results.[35]

3D-QSAR model was developed by phase module of Schrödinger. All dataset compounds were divided into a training set of 80% and a representative test set of 20%. Individual QSAR model of phase was used for construction of the simplest hypotheses that best correlates the experimental and predicted activities (Table 3). Statistically best QSAR model was generated with a $R^2 = 0.6283$, $F = 20.3$, SD = 0.1506, RMSE = 0.08 and $Q^2 = 0.6278$. For the highly active compound **4d**, the QSAR feature is shown in Figure 6A. Accumulation of highest positive coefficient (1.1×10^{-3})threshold of cyan colored hydrophobic or non polar cubes at the *ortho* position of **4d** infers the importance of substitution with hydrophobic moieties at *ortho* position rather than *para* and *meta* positions as shown in Figure 6B.

In the present study, the experimental procedure is operationally easy and the application of microwave irradiation leads to high yields in short reaction time. The synthesized acridinediones as SIRT1 inhibitors were evaluated by biochemical enzymatic assay and cell-based assay supported by *in silico* data. The compound (Z)-2-(5-bromo-2-hydroxybenzylidene) benzofuran-3(2*H*)-one was found to be a potential molecule inhibiting SIRT1.

Acknowledgments

The authors are thankful to the Department of Biotechnology (BT/PR13326/BRB/10/719/2009), Government of India, for their financial assistance. We thank Dr. Srikanth Viswanadha and Sumalatha from Incozen Therapeutics (P) Ltd for providing cell culture facilities.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.03.030.

References and notes

1. Yamamoto, H.; Schoonjans, K.; Auwerx, J. *Mol. Endocrinol.* **2007**, *21*, 1745.
2. Elliott, P. J.; Jirousek, M. *Curr. Opin. Investig. Drugs* **2008**, *9*, 371.
3. Jiang, W. J. *Biochem. Biophys. Res. Commun.* **2008**, *373*, 341.
4. Milne, J. C.; Denu, J. M. *Curr. Opin. Chem. Biol.* **2008**, *12*, 11.
5. Taylor, D. M.; Maxwell, M. M.; Luthi-Carter, R.; Kazantsev, A. G. *Cell. Mol. Life Sci.* **2008**, *65*, 4000.
6. Michishita, E.; Park, J. Y.; Burneskis, J. M.; Barrett, J. C.; Horikawa, I. *Mol. Bio. Cell* **2005**, *16*, 4623.
7. Luo, J.; Nikolaev, A. Y.; Imai, S.; Chen, D.; Su, F.; Shiloh, A.; Guarente, L.; Gu, W. *Cell* **2001**, *107*, 137.
8. Vaziri, H.; Dessain, S. K.; Ng Eaton, E.; Imai, S. I.; Frye, R. A.; Pandita, T. K.; Guarente, L.; Weinberg, R. A. *Cell* **2001**, *107*, 149.
9. Langley, E.; Pearson, M.; Faretta, M.; Bauer, U. M.; Frye, R. A.; Minucci, S.; Pelicci, P. G.; Kouzarides, T. J. *EMBO* **2002**, *21*, 2383.
10. 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.
11. Pagans, S.; Pedal, A.; North, B. J.; Kaehlcke, K.; Marshall, B. L.; Dorr, A.; Hetzer-Egger, C.; Henklein, P.; Frye, R.; McBurney, M. W.; Hruby, H.; Jung, M.; Verdin, E.; Ott, M. *PLoS Biol.* **2005**, *3*, e41.
12. Nemoto, S.; Fergusson, M. M.; Finkel, T. *J. Biol. Chem.* **2005**, *280*, 16456.
13. Rodgers, J. T.; Lerin, C.; Haas, W.; Gygi, S. P.; Spiegelman, B. M.; Puigserver, P. *Nature* **2005**, *434*, 113.
14. Hallows, W. C.; Lee, S.; Denu, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 10230.
15. Michan, S.; Sinclair, D. *Biochem. J.* **2007**, *404*, 1.
16. Wong, S.; Weber, J. D. *Biochem. J.* **2007**, *407*, 451.
17. Ford, J.; Jiang, M.; Milner, J. *Cancer Res.* **2005**, *65*, 10457.
18. Neugebauer, R. C.; Sippl, W.; Jung, M. *Curr. Pharm. Des.* **2008**, *14*, 562.
19. 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.
20. Bedalov, A.; Gatbonton, T.; Irvine, W. P.; Gottschling, D. E.; Simon, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 15113.
21. Posakony, J.; Hirao, M.; Stevens, S.; Simon, J. A.; Bedalov, A. *J. Med. Chem.* **2004**, *47*, 2635.
22. Neugebauer, R. C.; Uchiechowska, U.; Meier, R.; Hruby, H.; Valkov, V.; Verdin, E.; Sippl, W.; Jung, M. *J. Med. Chem.* **2008**, *51*, 1203.
23. 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.
24. Suzuki, T.; Imai, K.; Nakagawa, H.; Miyata, N. *ChemMedChem* **2006**, *1*, 1059.
25. Gey, C.; Kyrylenko, S.; Hennig, L.; Nguyen, L. H.; Buttner, A.; Pham, H. D.; Giannis, A. *Angew. Chem. Int. Ed.* **2007**, *46*, 5219.
26. Tervo, A. J.; Suuronen, T.; Kyrylenko, S.; Kuusisto, E.; Kiviranta, P. H.; Salminen, A.; Leppanen, J.; Poso, A. *J. Med. Chem.* **2006**, *49*, 7239.
27. Tervo, A. J.; Kyrylenko, S.; Niskanen, P.; Salminen, A.; Leppanen, J.; Nyronen, T. H.; Jarvinen, T.; Poso, A. *J. Med. Chem.* **2004**, *47*, 6292.
28. Kiviranta, P. H.; Leppanen, J.; Rinne, V. M.; Suuronen, T.; Kyrylenko, O.; Kyrylenko, S.; Kuusisto, E.; Tervo, A. J.; Jarvinen, T.; Salminen, A.; Poso, A.; Wallen, E. A. *Bioorg. Med. Chem. Lett* **2007**, *17*, 2448.
29. Kiviranta, P. H.; Leppanen, J.; Kyrylenko, S.; Salo, H. S.; Lahtela-Kakkonen, M.; Tervo, A. J.; Wittekindt, C.; Suuronen, T.; Kuusisto, E.; Jarvinen, T.; Salminen, A.; Poso, A.; Wallen, E. A. *J. Med. Chem.* **2006**, *49*, 7907.
30. Grozinger, C. M.; Chao, E. D.; Blackwell, H. E.; Moazed, D.; Schreiber, S. L. *J. Biol. Chem.* **2001**, *276*, 38837.
31. Autiero, I.; Costantini, S.; Colonna, G. *PloS ONE* **2009**, *4*, e7350.
32. Laskowski, R. A.; MacArthur, W. M.; Moss, D. S.; Thornton, J. M. J. *Appl. Crystallogr* **1993**, *26*, 283.
33. Thimmappa, H. M.; Yogeeswari, P.; Sriram, D. *Bioorg. Med. Chem. Lett* **2011**, *21*, 2125.
34. *(Z)-2-(5-bromo-2-hydroxybenzylidene) benzofuran-3(2H)-one Compound* ***4d***; 1H NMR (300 MHz, $CDCl_3$): $\delta = 0.92$ (s, 6H, $2*CH_3$), 0.99 (s, 6H, $2*CH_3$) 1.92 (d, $J = 9.1$ Hz, 2H), 2.07 (d, $J = 9.3$ Hz, 2H,), 2.41 (dd,, $J_{Gem} = 8.9$ Hz, 4H, (2∗CH2)H2, H7), 4.84 (s, 1H, H9), 5.2 (s, 2H,–O– CH2) 6.7–6.96 (m, 4H, ArH of phenyl at C9), 7.2–7.4 (m, 5H, ArH of benzyl), 8.4-9.3(m, 3H, ArH of benzoyl), 8.2 (s, 1H, NH); ^{13}C NMR (75 MHz, CDCl3): $\delta = 195.6, 163.8, 158.5, 153.5, 147.6, 143.2, 141.2, 134.2, 129.8, 128.3, 127.7, 127.0, 126.5, 121.1, 120, 116.4, 112.8, 111, 73.16, 52.3, 38.7, 32, 30.3, 27.1$; EI-MS m/z 665.3 $(M+H)^+$;Anal calcd. for $C_{37}H_{36}N_4O_8$: C, 66.86; H, 5.46; N, 8.43. Found: C, 66.91; H, 5.41; N, 8.47.
35. Arunasree, M. K.; Mallika, A.; Badiger, J.; Alinakhi; Pinaki, T.; Sachchidanand *Biochem. Biophys. Res. Commun.* **2010**, *401*, 13.
36. Lara, E.; Mai, A.; Calvanese, V.; Altucci, L.; Lopez-Nieva, P.; Martinez-Chantar, M. L.; Varela-Rey, M.; Rotili, D.; Nebbioso, A.; Ropero, S.; Montoya, G.; Oyarzabal, J.; Velasco, S.; Serrano, M.; Witt, M.; Villar-Garea, A.; Imhof, A.; Mato, J. M.; Esteller, M.; Fraga, M. F. *Oncogene* **2009**, *28*, 781.
37. Suzuki, T.; Imai, K.; Imai, E.; Iida, S.; Ueda, R.; Tsumoto, H.; Nakagawa, H.; Miyata, N. *Bioorg. Med. Chem.* **2009**, *17*, 5900.