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Amberlite IR-120H catalyzed MCR: Design, synthesis and crystal structure analysis of 1,8-dioxodecahydroacridines as potential inhibitors of sirtuins

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ABSTRACT

A rapid, inexpensive and high yielding method has been developed for the synthesis of 1,8-dioxodecahydroacridines using Amberlite IR-120H as a reusable catalyst under open air. These compounds were designed as potential inhibitors of sirtuins and prepared via the MCR of 5,5-dimethyl-1,3-cyclohexanedione, (hetero)aryl aldehydes and (hetero)aromatic amines under mild conditions. Further structure elaboration of a representative compound was performed via Pd catalyzed C–C bond forming reactions. The crystal structure analysis and H-bonding patterns along with in vitro inhibitory activity against yeast Sir2 of the same compound is presented. Docking studies indicated that the compound interacts well with the yeast Sir2.

The identification of suitable drug targets is one of the major challenges in cancer drug discovery. In the recent years, majority of the efforts have been devoted by focusing on signaling molecules mostly kinases as drug targets. However, with the emergence of kinase inhibitor resistant cancers, there is a growing need to identify newer targets and develop new drugs. Recently, inhibition of sirtuins has been described as a new approach for the discovery of novel anticancer drugs. 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.[1] 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-B, forkhead proteins etc.[2] Since sirtuins are up-regulated in many cancers hence they are considered as important targets for cancer therapeutics. Indeed, 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[3] 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. Recently we have reported the synthesis of 1,8-dioxo-octahydroxanthenes (**A**, Fig. 1) as potential anticancer agents.[4] In continuation of that work and our interest in the identification of inhibitors of sirtuins[5] we now report the

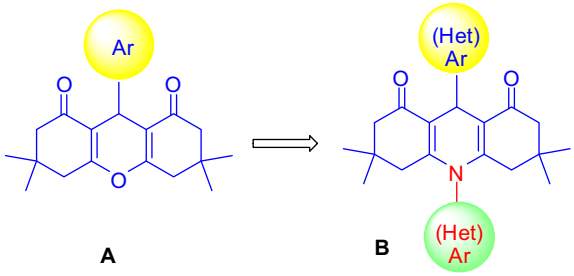

Figure 1. Novel 1,8-dioxodecahydroacridine derivatives (**B**) derived from **A**.

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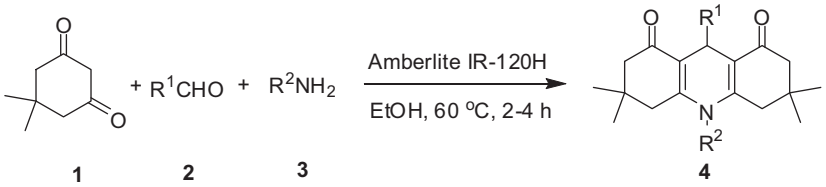
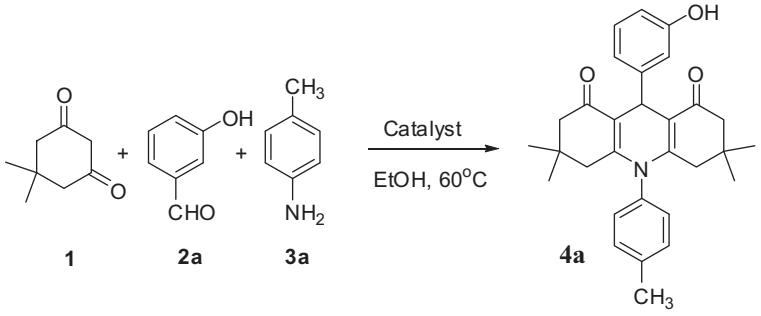


Scheme 1. Amberlite IR-120H catalyzed synthesis of 1,8-dioxodecahydroacridines.

Table 1
Effect of catalysts on the MCR of **1**, **2a** and **3a**[a]



Entry	Catalyst (amount)	Time (h)	Yield[b] (%)
1	Con. HCl (1–2 drops)	5	88
2	*p*-TSA (30 mg)	5	62
3	Amberlyst-15 (50 mg)	4	80
4	Amberlite IR-120H (50 mg)	2	95[c] (91, 87)
5	Amberlite IR-120H (50 mg)	1	72
6	No catalyst	5	0
7	Amberlite IR-120H (50 mg)	4	58[d]
8	Amberlite IR-120H (50 mg)	4	67[e]
9	Amberlite IR-120H (50 mg)	2	62[f]

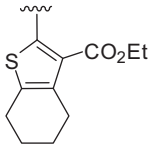
[a] All the reactions were carried out by using **1** (2 mmol), **2a** (1 mmol), **3a** (1 mmol) and a catalyst in EtOH (4 mL) at 60 °C under open air.
[b] Isolated yields.
[c] Catalyst was reused for additional two runs and figures within parentheses indicate the corresponding yield for each run.
[d] DMF was used in place of EtOH.
[e] MeCN was used in place of EtOH. The product isolated was found to be 9-(3-hydroxyphenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1*H*-xanthene-1,8(2*H*)-dione (**4aa**) instead of **4a** in this case.
[f] Dichloromethane was used in place of EtOH.

synthesis and sirtuin inhibiting properties of analogous 1, 8-dioxodecahydroacridine derivatives (**B**, Fig. 1) the design and selection of which was further supported by the docking studies (see later for a discussion).

The synthesis of 1,8-dioxodecahydroacridines is generally carried out by using a multi-component reaction (MCR) of dimedone, aldehydes, and different anilines or ammonium acetate in the presence of a range of catalysts for example *p*-dodecylbenezenesulfonic acid (DBSA),[6] $[B(C_6F_5)_3]$,[7] proline,[8] carbon-based solid acid (CBSA),[9] NH_4Cl,[10] Brønsted acidic imidazolium salts[11] and ceric ammonium nitrate (CAN).[12] Synthesis of these compounds by the classical Hantzsch's procedure[13] or reaction of aldoximes with dimedone, under microwave irradiation have also been reported.[14] The use of a resin based catalyst for example Amberlyst-15 was also found to be effective.[15] While, many of these methods are quite effective and can be performed under environmental friendly conditions we were in search for a resin based inexpensive, faster, operationally simple and high yielding method for the synthesis of our target compound **B**. We have found that the rapid synthesis of **B** (or **4**, Scheme 1) can be carried out in high yields by using Amberlite IR-120H as an inexpensive and recyclable catalyst[16] in the MCR of 5,5-dimethylcyclohexane-1,3-dione (**1**), aldehyde (**2**), and aniline (**3**) (Scheme 1). To the best of our knowledge this is the first example of synthesis of compound **B** (or **4**) catalyzed by Amberlite IR-120H. The preliminary results of this study are presented.

Initially, we carried out the reaction of diketone **1**, 3-hydroxybenzaldehyde (**2a**), and *p*-toluidine (**3a**) in EtOH at 60 °C in the presence of con. HCl and *p*-TSA when the desired product **4a** was isolated in 88% and 62% yield, respectively (entry 1 and 2, Table 1). However, to identify an environmental friendly condition the same reaction was performed in the presence of known catalyst Amberlyst-15 when the reaction was completed within 4 h affording **4a** in 80% yield (entry 3, Table 1). The use of another resin based catalyst for example Amberlite IR-120H was found to be more effective as the reaction was completed within 2 h affording **4a** in 95% yield (entry 4, Table 1). A further decrease in reaction time decreased the product yield (entry 5, Table 1) whereas the reaction did not proceed in the absence of catalyst indicating the key role played by the catalyst in the present MCR. To assess the recyclability of Amberlite IR-120H the catalyst was recovered by filtration (followed by washing with EtOAc and drying) and reused when **4a** was isolated without significant loss of its yield (entry 4, Table 1). While all these reactions were performed in EtOH the use of other solvents for example DMF (entry 7, Table 1), MeCN (entry 8, Table 1), dichloromethane (entry 9, Table 1) etc. was also explored and found to be less effective. In case of MeCN a different product that is 9-(3-hydroxyphenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1*H*-xanthene-1,8(2*H*)-dione (**4aa**) was isolated instead of **4a** whereas a mixture of both was formed when water was used as a solvent.

Table 2
Synthesis of 1,8-dioxodecahydroacridines using Amberlite IR-120H (Scheme 1)[a]

Entry	Aldehyde;**2**; R^1 =	Aniline;**3**; R^2 =	Time (h)	Product (**4**)	Yield[b] (%)
1	**2a**; 3-OHC_6H_4	**3a**; 4-Me C_6H_4	3	**4a**	91
2	**2a**	**3b**; 4-BrC_6H_4	3	**4b**	89
3	**2a**	**3c**; 4-IC_6H_4	4	**4c**	94
4	**2a**	**3d**; 2-IC_6H_4	3	**4d**	90
5	**2a**	**3e**; 4-FC_6H_4	2	**4e**	95
6	**2b**; 3-$NO_2C_6H_4$	**3c**	4	**4f**	86[c]
7	**2b**	**3f**; 4-ClC_6H_4	2	**4g**	88
8	**2c**; 2-OHC_6H_4	**3h**; C_6H_5	3	**4h**	85
9	**2a**	**3i**; 4-$MeCOC_6H_4$	4	**4i**	92
10	**2a**	**3j**; 4-CNC_6H_4	3	**4j**	85
11	**2a**	**3k**; 4-$CF_3C_6H_4$	4	**4k**	88
12	**2d**; 2-Naphthyl	**3b**	3	**4l**	90[c]
13	**2e**; 2-Thienyl	**3c**	3	**4m**	87
14	**2e**		4	**4n**	87[c]
15	**2f**; 3-FC_6H_4	**3e**	3	**4o**	88
16	**2g**; C_6H_5	**3a**	3	**4p**	73

[a] All the reactions were carried out by using 5,5-dimethyl-1,3-cyclohexanedione **1** (2 mmol), aldehyde **2** (1 mmol), amine **3** (1 mmol) and Amberlite IR-120H (50 mg) in EtOH (4 mL) at 60 °C under open air.
[b] Isolated yields.
[c] After purification by column chromatography.

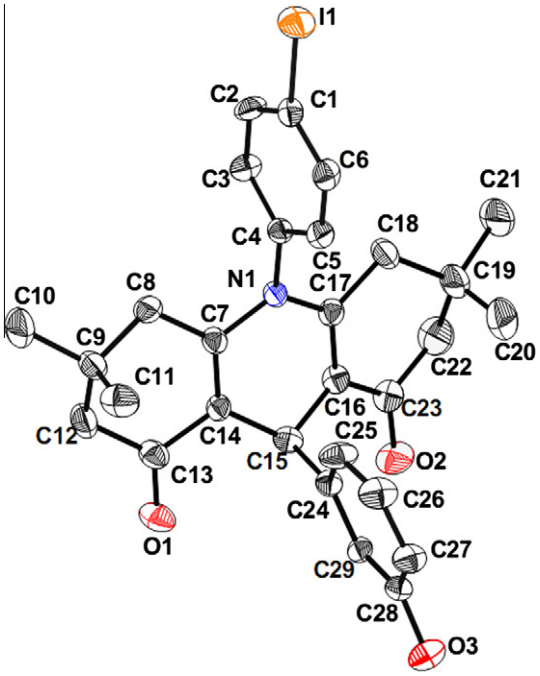


Figure 2. Thermal ellipsoidal plot compound **4c** (30% probability, hydrogen atoms and another asymmetric molecule has been omitted for clarity).

Encouraged by the utility of Amberlite IR-120H as a catalyst in the present MCR in EtOH various functionalized 1, 8-dioxodecahydroacridines (**4**) were prepared by using structurally diverse aromatic aldehydes (**2**) and amines (**3**) bearing various electron donating and electron withdrawing groups (Table 2). Both aryl and heteroaryl aldehydes as well as amines were found to be effective in this reaction. While all the reactants used in the present MCR are commercially available the amine that is ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate **3d** (entry 14, Table 2) used were prepared according to the reported method.[17]

All the compounds (**4a–4p**) synthesized were characterized by spectral data (^1H and ^{13}C NMR, IR, MS and HPLC) and the molecular structure of a representative compound **4c** was confirmed unambiguously by single crystal X-ray diffraction studies (Fig. 2).[18] Further, crystal structure analysis was carried out to understand the packing and/or hydrogen bonding patterns in crystal of this molecule and results are summarized in the following section.

The crystal structure analysis of **4c** indicated supramolecular interactions between carbonyl oxygen (C=O) and methylene hydrogen (CH_2) that were within the range of 2.459–2.503 Å. Since only two supramolecular interactions (O4···H22B = 2.502 Å and O4···H8A = 2.460 Å) were present between hydrogen and oxygen, hence it gave very short network as shown in Figure 3. These supramolecular interactions were within the range of van der Waals radii.[19]

Having prepared the iodo derivative **4c** we then focused on further structure elaboration of this compound by using various Pd-catalyzed C–C bond forming reactions such as Sonogashira, Suzuki, and Heck coupling (Scheme 2).

Some of 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) Sir2 protein (a yeast homologue of mammalian SIRT1). Splitomicin,[20a] a known inhibitor of sirtuin, was used as a reference compound in this assay. Compounds (**4**) were tested for their ability to inhibit Sir2 protein by estimating inhibition of growth of yeast strain containing URA3 gene at telomeric locus, in presence of 5-fluoroorotic acid (5-FOA).[20b] 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 URA3 was inserted in the silenced telomeric region where it is silenced by yeast Sir2 protein (Scheme 3). Inhibition of Sir2 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. Among all the compounds tested (see ESI) the compound **4c** showed ~40% inhibition of Sir2. The other compounds that showed inhibition of Sir2

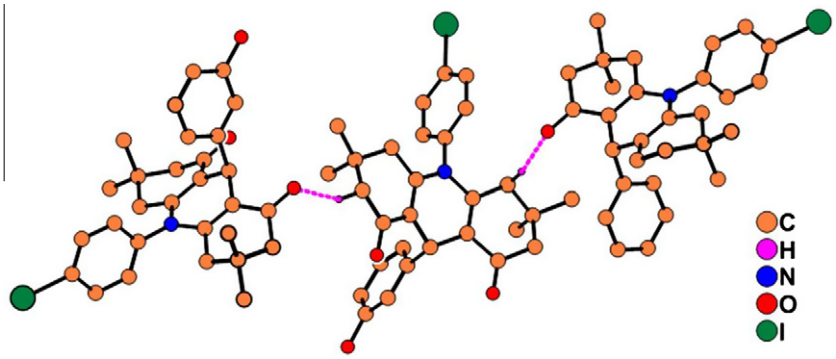
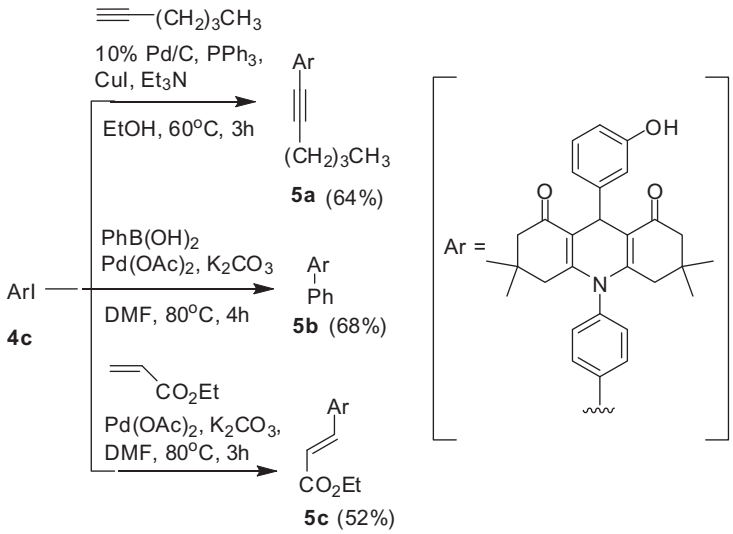
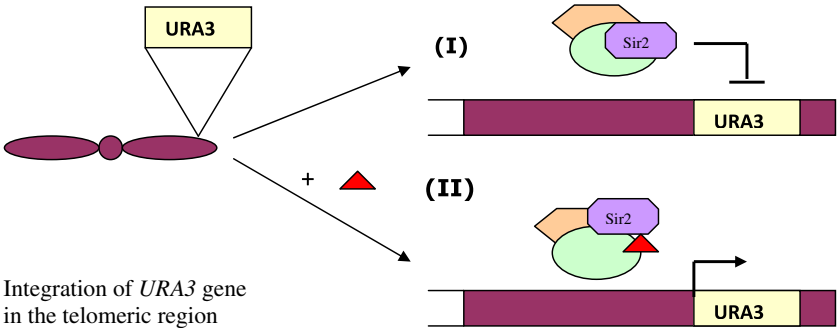


Figure 3. The supramolecular interactions between hydrogen and oxygen in compound **4c**.



Scheme 2. Structure elaboration of **4c** via Pd catalyzed C–C bond forming reactions.



Scheme 3. Cell based Sir2 mediated reporter silencing assay in yeast. (I) Growth in presence of 5-fluoroorotic acid (5-FOA): Sir2 mediated silencing of URA3 gene permits growth in FOA. (II) No growth in presence of FOA: Inhibition of Sir2 results in expression of URA3 gene and cell death in presence of FOA.

includes **4a** (30%), **4b** (32%), **4e** (33%) and **4f** (35%) indicating a polar group preferably an OH at the *m*-position of the 9-phenyl ring was beneficial for Sir2 inhibition. Similarly, a substituent preferably a halo group at the *p*-position of the 10-phenyl ring seemed to be favorable for inhibitory effect.

To understand the binding mode of the compound **4c** with yeast Sir2 protein docking[21] was carried out using the crystal structure of yeast Sir2 (PDB ID: 1Q1A). The lowest energy conformation was selected and the ligand interactions (H-bonding and hydrophobic interaction) with the active sites of yeast Sir2 were determined. Two H-bond interactions were observed between Asp 43 and the molecule **4c** involving (i) one of the carbonyl group of **4c** with NH of Asp 43 and (ii) hydroxyl group of **4c** with OH of COOH in Asp 43 (Fig. 4). The presence of *N*-aryl group facilitated **4c** to adopt the favorable conformation suitable for binding with Sir2 protein. The glide score obtained for **4c** including other parameters are listed in Table 3.

In conclusion, a rapid, inexpensive and high yielding method has been developed for the synthesis of 1,8-dioxodecahydroacridines using Amberlite IR-120H as a recyclable catalyst under open air. To the best of our knowledge this is the first example of synthesis of these compounds catalyzed by Amberlite IR-120H. These acridine derivatives were designed as potential inhibitors of sirtuins (that are considered as emerging targets in cancer drug discovery)

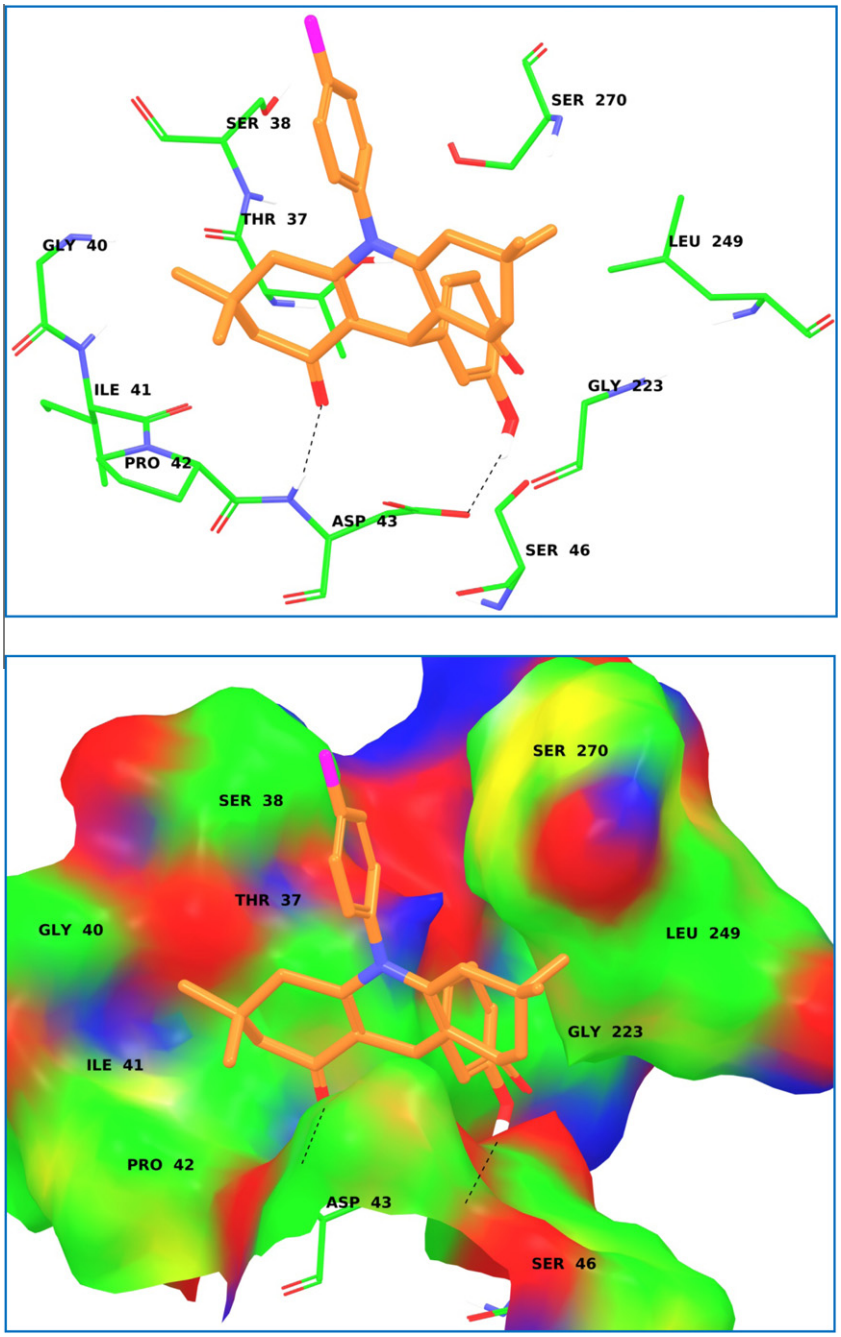


Figure 4. Binding mode and interactions orientation of **4c** with Yeast Sir2 (PDB ID: 1Q1A).

Table 3
Glide score and contributing parameters

Molecule	GScore	LipophilicEvdW	HBond	Electro	PhobicPenal
4c	−3.0	−2.35	−0.48	−0.52	0.34

LipophilicEvdW: Chemscore lipophilic pair term and fraction of the total protein–ligand vdw energy.
HBond: Rewards for hydrogen bonding interaction between ligand and protein.
Electro: Electrostatic reward.
PhobicPenal: Penalty for solvent-exposed ligand groups.

and prepared via the MCR of 5,5-dimethyl-1,3-cyclohexanedione, (hetero)aryl aldehydes and (hetero)aromatic amines under mild conditions. Further structure elaboration of a representative compound was performed via Sonogashira, Suzuki and Heck reactions. The crystal structure analysis and H-bonding patterns along with in vitro inhibitory activity against yeast Sir2 of the same compound is presented. Docking studies indicated that the compound interacts well with yeast Sir2. Overall, the Amberlite IR-120H catalyzed MCR described here could be useful in constructing library of small molecules based on 1,8-dioxodecahydroacridine framework leading to the identification of novel inhibitors of sirtuins.

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.2013.01.026.

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