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Amberlyst-15 catalysed sonochemical synthesis of 2-amino-4,6-disubstituted nicotinonitrile derivatives and their biological evaluation

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ABSTRACT

The 2-amino nicotinonitrile framework has been explored first time for the identification of potential inhibitors of SIRT1. Thus a series of targeted 2-amino-4,6-disubstituted nicotinonitrile derivatives were synthesized by employing an ultrasound assisted MCR of ketones, aldehydes, malononitrile and ammonium acetate. The MCR was carried out in the presence of Amberlyst-15 in MeCN under mild conditions to give the desired product in good yields. The reaction was less efficient in the absence of air whereas combination of Amberlyst-15, ultrasound, air and MeCN was essential for the success of this MCR. Several of the synthesized compounds showed good activities when tested for their SIRT1 inhibitory potential *in vitro* among which **5c, 5e** and **5n** were identified as the most potent (IC_{50} ~ 3 μM) and were better than the known inhibitor nicotinamide (IC_{50} ~109 μM). In the *in silico* docking studies these three compounds showed better binding energy (> 100 kcal/mol) and higher number of interactions than nicotinamide (binding energy -88.38 kcal/mol). While both amino (–NH_2) and cyano (–CN) groups of nicotinonitrile derivatives formed H-bonds with the ASN346 and HIS363 residue respectively the nicotinamide showed similar interactions with ASP348 and ILE347 through its amide (–$CONH_2$) moiety. Compound **5c, 5e** and **5n** has been identified as initial hits for further study.

1. Introduction

Compounds containing the 2-amino nicotinonitrile **A** (or 2-amino-3-cyanopyridine, Fig. 1) framework are known to possess numerous pharmacological activities [1–4] including anticancer as well as antitumor properties [5,6]. Indeed, this framework seemed to play crucial roles for various pharmacological functions and hence attracted considerable interest in medicinal and pharmaceutical chemistry. In spite of a number of reports on their anticancer activities [7–10], none of the 2-amino nicotinonitrile derivatives have been reported to enter into the any phase of clinical studies till date as potential anticancer agents. Moreover, the structure–activity relationship (SAR) was not clearly elaborated in some of these studies though the overall findings were promising or remarkable in several cases. Further, the thorough studies on molecular interactions of 2-amino nicotinonitrile based anticancer agents with their pharmacological targets via *in silico* or similar approaches are not common in the literature. Hence it was desirable to adopt a fresh Med Chem approach for studying anticancer properties of this class of compounds. This is particularly desirable from

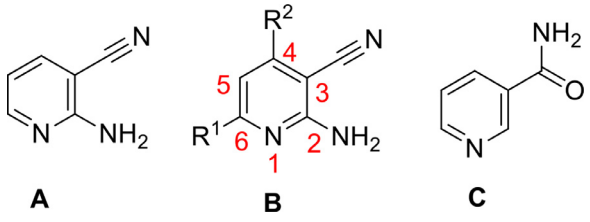

Fig. 1. 2-Amino nicotinonitrile **A**, the template **B** and nicotinamide **C**.

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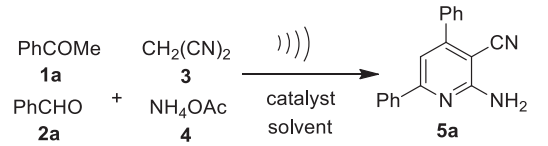
the view point of the fact that identification of new drugs is one of the key solutions to the multifaceted problem of drug resistance [11]. Notably, drug resistance often causes a major hurdle resulting in failure of chemotherapy based approaches on several occasions. Due to our interest in bioactive nicotinonitriles [12] herein we report the synthesis, *in vitro* and *in silico* evaluation of a series of compounds based on the template **B** (Fig. 1) derived from **A**. We have chosen SIRT1 as the pharmacological target for our study because (i) being considered as important targets for cancer therapeutics sirtuins (class III NAD-dependent deacetylases) are shown to up-regulated in various types of cancer [13] whereas (ii) inhibition of sirtuins allows re-expression of silenced tumor suppressor genes, leading to the decreased growth of cancer cells. Moreover, the rational for choosing SIRT1 as a potential pharmacological target for the nicotinonitrile derivatives based on **B** stem from the fact that the structurally similar nicotinamide **C** (Fig. 1) is one of the earliest inhibitors of sirtuins including SIRT1 [14]. While the template **B**, as shown in Fig. 1, has three centers (e.g. C-4, C-5 and C-6) for the introduction of diversity into the central pyridine ring, we initially focused on the modification of C-4 and C-6 substituents. This is because the nature and type of substituents present at these positions were reported to play key roles in biological activities. Hence, we aimed to examine the effect of variation of C-4 and C-6 groups on biological activities of such derivatives and consequently, we required a quick access to 2-amino-4,6-disubstituted nicotinonitrile derivatives based on template **B**.

2. Results and discussion

Due to their importance not only in the area of medicinal / pharmaceutical chemistry but also as valuable precursors in organic synthesis a diverse range of methods have been reported for the synthesis of 2-amino nicotinonitrile derivatives [15]. Among them the multicomponent reaction (MCR) of aldehydes, ketones, malononitrile, and ammonium acetate has become the most common route towards the synthesis of this class of compounds. A wide range of reaction conditions have been reported for this MCR of which some selected examples include the use of (i) catalysts such as $Yb(PFO)_4$ [16], MgO [17], graphene oxide [18], silica nanoparticle [19], $Bi(NO_3)_2{\bullet}5H_2O$ [20] etc., or (ii) ionic liquid e.g. 1-butyl-3-methylimidazolium tetrafluroroborate ([Bmim][BF_4]) [21] or (iii) only solvent such as polyethylene glycol (PEG) [22]. The use of microwave [23,24] and ultrasound irradiation [25] has also been reported for the faster synthesis of this class of compounds. Indeed, the ultrasound-assisted synthesis of 2-amino nicotinonitrile particularly attracted our attention [25] because the methodology was free from the use of any catalyst and organic media. Moreover, the MCR proceeded at 50 °C in pure water affording the desired products in good yield (75–97%). All these features encouraged us to adopt this sonochemical protocol for the synthesis of our target compounds based on **B**. However, in our effort we encountered with some difficulties like sluggishness of the reaction thereby requirement of either longer reaction time or applying higher temperature. In some cases, the reaction afforded the intermediate product rather than the desired compound or did not proceed at all. The reason for such observation appeared to be partially due to the poor solubility of reactants especially the acetophenones in pure water. Indeed, we noted that generally acetophenones containing a phenolic hydroxyl group were used in the previous study that perhaps aided their solubility in pure water. It was therefore necessary to establish an appropriate reaction condition that could serve our purpose. The previous reports on successful use of acidic catalysts in the synthesis of 2-amino nicotinonitriles prompted us to explore the use of Amberlyst-15 for accessing the compounds based on **B**. As a low-cost and readily available heterogeneous catalyst Amberlyst-15 gained our attention [26–30] due to its non-hazardous nature and easy removal from the reaction mixture, for example, via simple filtration [31]. We have observed that the use of Amberlyst-15 not only allowed the reaction to proceed at room temperature but also reduced the reaction time though marginally. Thus the ultrasound assisted MCR of ketones (**1**), aldehydes (**2**), malononitrile (**3**), and ammonium acetate (**4**) was carried out in the presence of Amberlyst-15 in MeCN under mild conditions to give the desired product **5** in good yields (Scheme 1). Notably, the use of Amberlyst-15 for the rapid synthesis of **5** is not known in the literature.

Table 1
Effect of reaction conditions on the MCR of **1a, 2a, 3** and **4**[a].

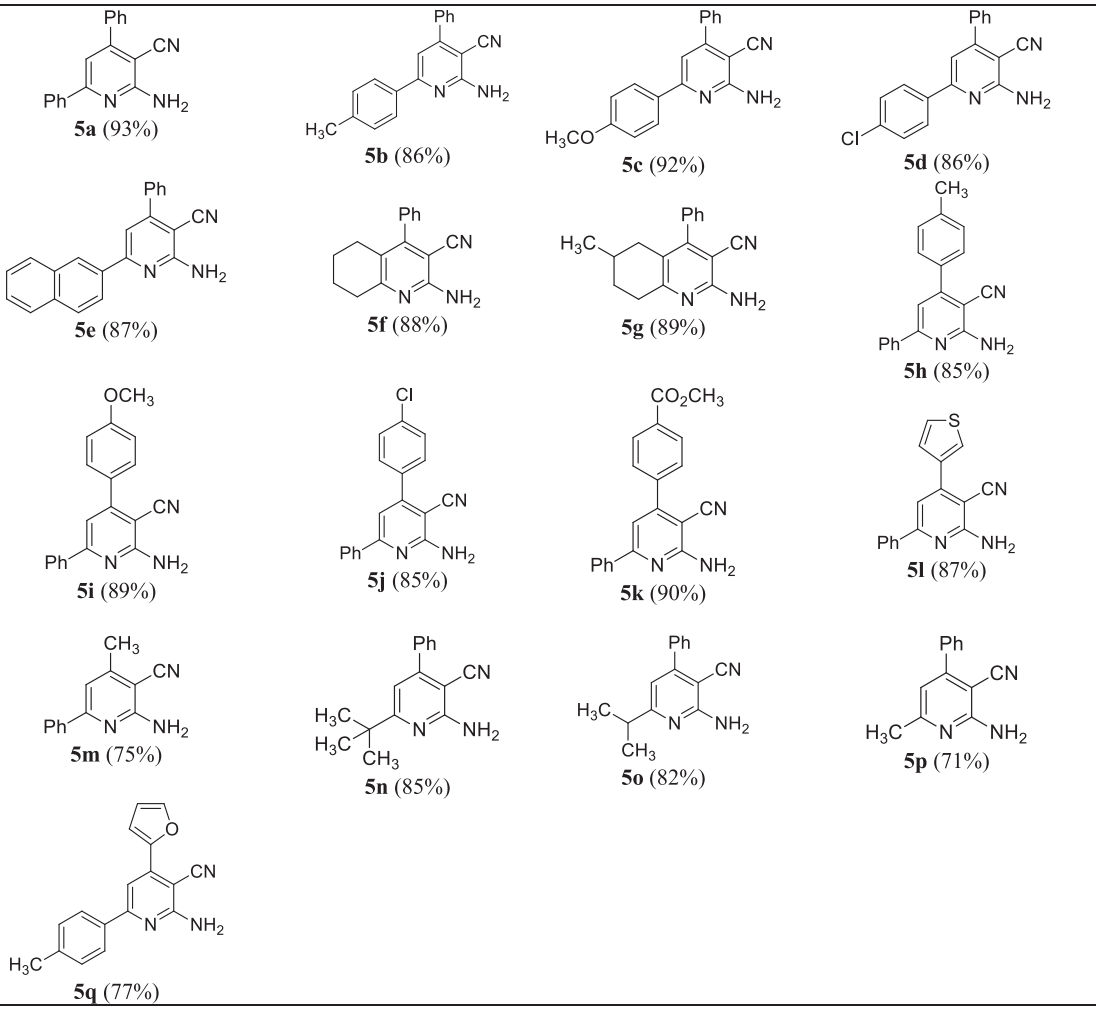


Entry	Catalyst	Solvent	T (°C)	Yield[b]
1.	No	No	100	88[c]
2.	No	Toluene	100	75[c]
3.	Amberlyst-15	Toluene	45–50	76[c]
4.	Amberlyst-15	Toluene	25–30	84
5.	Amberlyst-15	MeCN	25–30	93
6.	Amberlyst-15	MeCN	25–30	89[d]
7.	Amberlyst-15	EtOH	25–30	77
8.	Amberlyst-15	DCE	25–30	74
9.	Amberlyst-15	THF	25–30	61
10.	Amberlyst-15	No	25–30	60
11.	Amberlyst-15	MeCN	25–30	12[e]
12.	No	MeCN	25–30	49
13.	No	MeCN	25–30	27[c]
14	Amberlyst-15	MeCN	25–30	39[c]

[a] Reactions were carried out using acetophenone **1a** (1 mmol.), benzaldehyde **2a** (1 mmol), **3** (1 mmol), **4** (1 mmol) and a catalyst in a solvent (5 mL) under ultrasound irradiation for 10 min in the presence of air.
[b] Isolated yields.
[c] The reaction was performed in the absence of ultrasound for 3 h.
[d] The reaction was performed for 20 min.
[e] The reaction was performed under nitrogen atmosphere.

Initially, the MCR of acetophenone (**1a**), benzaldehyde (**2a**), malononitrile (**3**), and ammonium acetate (**4**) was carried out in the absence of any catalyst and solvent under conventional heating conditions in the presence of air (entry 1, Table 1). While the desired product **5a** was obtained in good yield however the reaction required maintaining a high temperature e.g. 100 °C and longer duration e.g. 3 h. The use of toluene (as a solvent) (entry 2, Table 1) or combination of toluene and Amberlyst-15 (as a catalyst) (entry 3, Table 1) did not change the situation significantly though the reaction temperature could be decreased drastically in the latter case. However, the use of ultrasound irradiation using a laboratory ultrasonic bath SONOREX SUPER RK 510H model producing irradiation of 35 kHz not only improved the product yield but also decreased the reaction temperature as well as time (entry 4, Table 1). Indeed, a further improvement in product yield was observed when the nonpolar solvent toluene was replaced by the relatively more polar solvent MeCN (entry 5, Table 1). Encouraged by this observation we carried out the reaction for a longer time i.e. 20 min however no significant improvement in product yield was observed (entry 6, Table 1). The use of more polar solvent such as EtOH or less polar solvents such as DCE (1,2-dichloroethane) or THF was examined and found to be less effective under the conditions employed (entries 7–9, Table 1). Indeed, the omission of solvent but not catalyst was also found to be less effective (entry 10, Table 1). To understand the role of air, the MCR was performed under nitrogen atmosphere when the desired product was isolated

Table 2
Amberlyst-15 catalyzed synthesis of 2-amino-4,6-disubstituted nicotinonitrile derivatives (**5**) under ultrasound irradiation[a,b] (Scheme 1).



[a] All reactions were carried out using the ketone **1** (1 mmol.), aldehyde **2** (1 mmol), **3** (1 mmol), **4** (1 mmol) and Amberlyst-15 (10% w/w) in MeCN (5 mL) at 25–30 °C under ultrasound irradiation for 10 min in the presence of air.
[b] Figure in the bracket represents isolated yield.

in poor yield (entry 11, Table 1) indicating the need of presence of air in the current transformation. Finally, the reaction was performed in the absence of catalyst in MeCN, in the absence of catalyst and ultrasound in MeCN and in the absence of ultrasound only (entries 12–14, Table 1). Results of all these studies suggested that the combination of Amberlyst-15, ultrasound, air and MeCN was most effective for the preparation of **5a** and therefore this combination was used for the preparation of its other related analogues (Scheme 1 and Table 2). A variety of ketones (**1**) including substituted acetophenones as well as 1-(naphthalen-2-yl)ethanone, cyclohexanones, aliphatic ketones etc. and aldehydes (**2**) including substituted benzaldehydes, heteroaryl aldehydes, acetaldehyde etc. were employed in this MCR under the optimized conditions. The reaction proceeded well irrespective of the nature of groups / substituents present in the ketone and aldehyde affording the desired 2-amino-4,6-disubstituted nicotinonitrile derivatives (**5**) in good yields.

All the 2-amino nicotinonitrile derivatives synthesized were characterized by spectral (NMR, MS) data. While the C-5 proton could be detected for most of the compounds (except **5f** and **5 g** that lack this proton) in their ^{1}HNMR spectra generally as a singlet in the range δ 7.5–7.0 depending on the substituent present at C-4 and C-6 position the NH_2 group appeared near δ 5.5 in

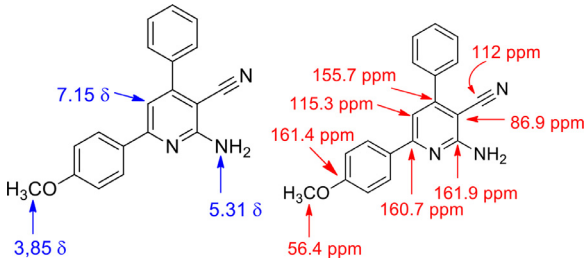


Fig. 2. Partial representation of ^{1}H and ^{13}C NMR spectral data of azaindole derivative **5c**.

$CDCl_3$ but at different value in DMSO-d_6. The presence of amine and CN moiety was also indicated by the IR absorptions near 3350 and 2200 cm^{-1} respectively. The partial ^{1}H and ^{13}C NMR data of a representative compound i.e. **5c** is shown in Fig. 2. The -OMe group appeared near δ 3.8 and 56.4 ppm in the ^{1}H and ^{13}C NMR spectra, respectively. Similarly, the proton and carbon at 5-position appeared near δ 7.2 and 115.3 ppm in the ^{1}H and ^{13}C NMR spectra, respectively. Moreover, the ^{13}C NMR spectra showed that the carbon at 2, 3, 4 and 6-position appeared near 161.9, 86.9, 155.7

Table 3
SIRT1 inhibition of compound **5** *in vitro*[a].

Compounds	Enzymatic assay % inhibition @ 10 μM[a]	IC_{50} (μM)
Control	0	–
Nicotinamide	–	109.2 ± 1.73
5a	38.9	–
5b	43.5	–
5c	79.2	2.78 ± 0.43
5d	49.8	–
5e	76.4	2.93 ± 0.12
5f	38.4	–
5g	37.8	–
5h	39.5	–
5i	69.2	3.45 ± 0.92
5j	42.7	–
5k	46.8	–
5l	65.8	3.57 ± 0.87
5m	28.4	–
5n	78.1	2.89 ± 0.51
5o	49.4	–
5p	26.8	–
5q	67.9	3.51 ± 0.75

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

and 160.7 ppm whereas the CN appeared at 112 ppm. The carbon bearing -OMe group appeared near 161.4 ppm.

A plausible reaction mechanism for the Amberlyst-15 catalyzed synthesis of 2-amino-4,6-disubstituted nicotinonitrile derivatives under ultrasound irradiation is presented in Scheme 2. The reaction proceeded *via* activating the aldehyde (**2**) and ketone (**1**) by the catalyst Amberlyst-15 to react with malononitrile and *in-situ* generated NH_3 [from ammonium acetate (**4**)] under ultrasound respectively. As a result of this the corresponding intermediate **E-1** and the imine was formed. Then **E-1** on reaction with the tautomeric form of imine afforded the intermediate **E-2** that on intramolecular cyclization assisted by ultrasound furnished **E-3**. Notably, the catalyst Amberlyst-15 was regenerated during this process. The tautomeric form of **E-3** then underwent aerial oxidation in the presence of aerial oxygen and Amberlyst-15 assisted by ultrasound to give the product **5**.

Next we choose to assess the inhibitory activities of synthesized 2-amino-4,6-disubstituted nicotinonitrile derivatives (**5**) against SIRT1 *in vitro*. The structurally similar nicotinamide, a known inhibitor of sirtuins including SIRT1 (the reported IC_{50} value against SIRT1 = 120 μM) [14] was used as a reference compound in this assay. Indeed, nicotinamide is known to block the proliferation and promote apoptosis in leukemic cells and reported to inhibit the growth and viability of human prostate cancer cells [32,33]. Nevertheless, all these compounds were tested at 10 μM using a reported biochemical enzymatic assay method [34]. The compounds that showed good activities in this assay (> 50% inhibition, Table 3) include **5c, 5e, 5i, 5l, 5n** and **5q** whereas compound **5b, 5d, 5j, 5k** and **5o** showed mediocre activities (> 40% inhibition, Table 3). While understanding a precise Structure-Activity-Relationship (SAR) within the current series of 2-amino-4,6-disubstituted nicotinonitrile derivatives was not straightforward the nature as well as type of substituents at C-4 and C-6 position appeared to play a key role in activities (Fig. 3). In general, arene (with a particular group) / heteroarene (e.g. thienyl and furan etc.) moieties were favored at these positions though a t-butyl group at C-6 position was also favored e.g. compound **5n**. Similarly, a bulky naphthyl ring at C-6 position was well tolerated e.g. compound **5e** whereas smaller substituents e.g. i-Pr or Me at this position was not favored. Among the groups attached to the arene ring the OMe substituent was found to be the most favored (e.g. **5c** and **5i**) over others e.g. Cl, Me CO_2Me etc. The compounds containing the pyridine ring fused with a cyclohexane moiety appeared to be less attractive in the current *in vitro* assay.

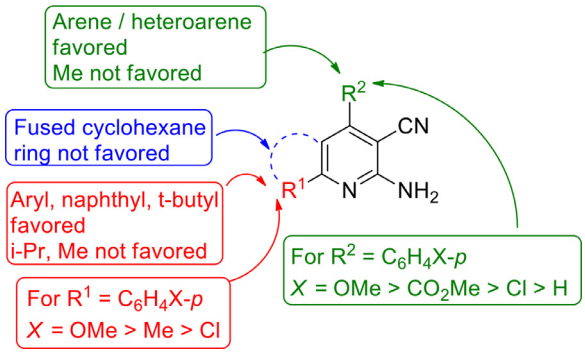


Fig. 3. Summary of SAR for SIRT1 inhibitory activities of 2-amino nicotinonitriles (**5**).

The compounds possessing good activities along with the reference compound nicotinamide were taken further for concentration dependent study and the corresponding IC_{50} values are presented in Table 3. While all these compounds appeared to be several fold potent than nicotinamide the compound **5c, 5e** and **5n** were identified as the most potent among them (IC_{50} ~ 3 μM). In order to understand their interactions with SIRT1 the *in silico* docking studies were carried out using these compounds including nicotinamide against the SIRT1 protein (PDB: 4I5I). The iGEMDOCK version2.1 software [35], a program for computing ligand conformation and orientation relative to the active site of the protein was used for the docking studies and results are presented in Table 4. All the test compounds showed binding activity with the catalytic residues of SIRT1. Indeed, the docking interactions occurred with the catalytic domain residues of 241–516. The compound **5c** and **5n** showed better binding energy than others that correlated their activity observed against SIRT1 *in vitro* (Table 3). Notably, nicotinamide showed binding energy lowest among them that also accounted its low potency compared to the current series of nicotinonitrile derivatives examined. Nevertheless, the amino (–NH_2) and cyano (–CN) groups of nicotinonitrile derivatives interacted with the ASN346 and HIS363 residue respectively through a H-bond in most of the cases including **5c** and **5n** (Figs. 4 and 5, see also Fig. S-1, 2, 3, 4, 5, 6 and 7, suppl data). These compounds also interacted with ALA262, ILE347, ILE270, HIS363, ILE411, PHE297 and PHE273 commonly through Van der Waals, pi-pi and pi-alkyl interactions in the catalytic domain of SIRT1. While similar interactions with ASP348 and ILE347 was shown by nicotinamide through its amide (–$CONH_2$) moiety (Fig. 6) the lower number of total interactions compared to nicotinonitrile derivatives was responsible for its lower binding energy thereby potency. Overall, the current series of nicotinonitrile derivatives especially the compound **5c** and **5n** are of further medicinal interest.

3. Conclusions

In conclusion, 2-amino nicotinonitrile framework was explored for the identification of potential inhibitors of SIRT1. Accordingly, the ultrasound assisted MCR was employed for the synthesis of a series of targeted 2-amino-4,6-disubstituted nicotinonitrile derivatives. The methodology involving the reaction of ketones, aldehydes, malononitrile and ammonium acetate was carried out in the presence of Amberlyst-15 in MeCN under mild conditions to give the desired product in good yields. The reaction was found to be less efficient in the absence of air whereas combination of Amberlyst-15, ultrasound, air and MeCN was found to be essential for the success of this MCR. Notably, the use of Amberlyst-15

Table 4
Summary of interactions of test compounds with SIRT1 *in silico*.

Compounds	Estimated Total Energy (kcal/mol)	Active site interacting residues
5c	−105.78	ASN346, HIS363, PHE273, PHE297, PHE414, ILE411, ILE347, ILE270
5e	−100.14	ASN346, HIS363, PHE297, PHE273, PHE414, ALA262, ILE270, ILE316, ILE347
5i	−97.03	ASN346, PHE273, ILE411, ILE316, ILE347, ILE270
5l	−95.52	HIS363, ILE347, ILE270, ILE411, VAL412, PHE273
5n	−103.91	ASN346, HIS363, PHE273,PHE297, ALA262, ILE347, ILE270, ILE411
5q	−96.94	ASN346, ALA262, PHE273, ILE347, ILE316, ILE270
Nicotinamide	−88.38	ASP348, ILE347, PHE273, ALA262, ILE279

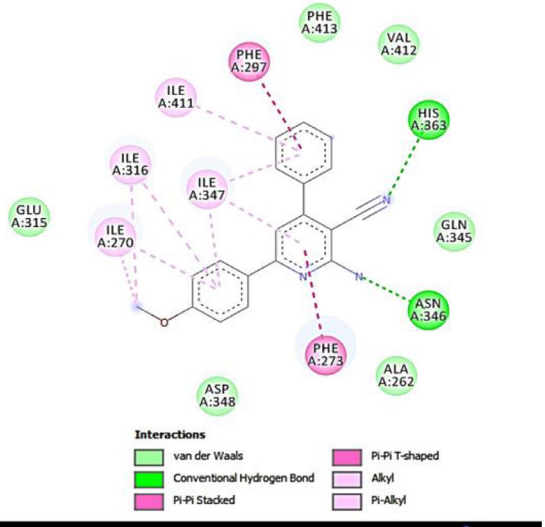


Fig. 4. Binding interactions and docked pose of compound **5c** at the catalytic site of SIRT1 (PDB: 4I5I).

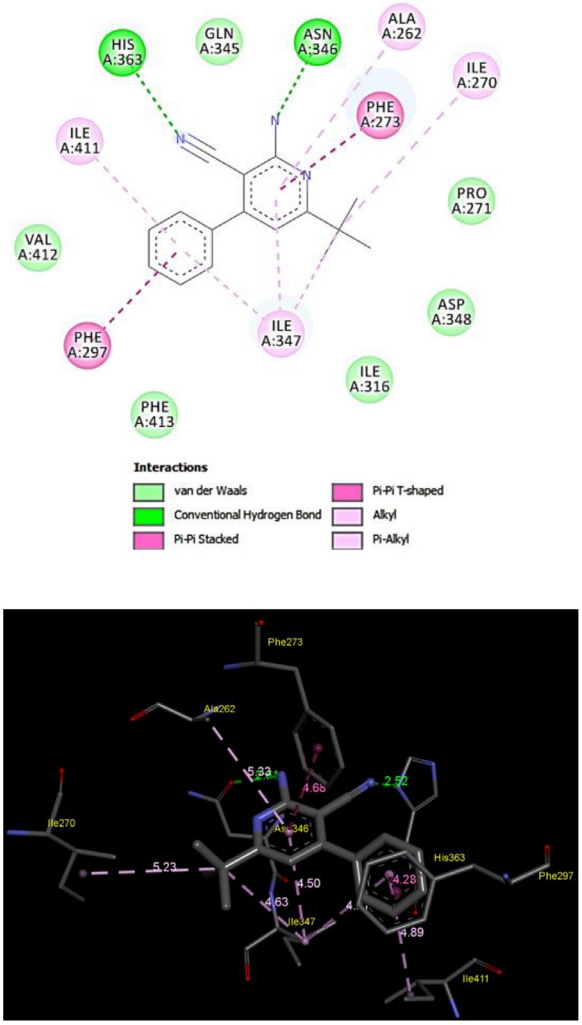


Fig. 5. Binding interactions and docked pose of compound **5n** at the catalytic site of SIRT1 (PDB: 4I5I).

for the rapid synthesis of 2-amino-4,6-disubstituted nicotinonitrile derivatives is not known in the literature. All the synthesized compounds were tested for their SIRT1 inhibitory potential *in vitro* and several of them showed good activities. The SAR study suggested that the inhibitory activity was dependent on the nature and type of substituents present at C-4 and C-6 positions. The compound **5c, 5e** and **5n** were identified as the most potent inhibitors (IC_{50} ~ 3 μM) and were better than the reference compound nicotinamide (IC_{50} ~109 μM). This was further supported by the *in silico* docking studies where these three compounds showed better binding energy (> 100 kcal/mol) and higher number of interactions than nicotinamide (binding energy −88.38 kcal/mol). While both amino (–NH_2) and cyano (–CN) groups of nicotinonitrile derivatives formed H-bonds with the ASN346 and HIS363 residue respectively the nicotinamide showed similar interactions with ASP348 and ILE347 through its amide (–$CONH_2$) moiety. Nevertheless, being potent inhibitors of SIRT1 the compound **5c, 5e** and **5n** has been identified as initial hits that deserves further study. Thus, the current study not only demonstrated the utility of Amberlyst-15 for the rapid synthesis of 2-amino-4,6-disubstituted nicotinonitrile derivatives but also indicated potential of 2-amino nicotinonitrile as a new framework for the identification of promising inhibitors of SIRT1.

Authors' statement

Chandra Sekhar Challa and Devanna Nayakanti were involved in the investigation, methodology development and formal analysis related related to all the target compounds presented in the current manuscript.

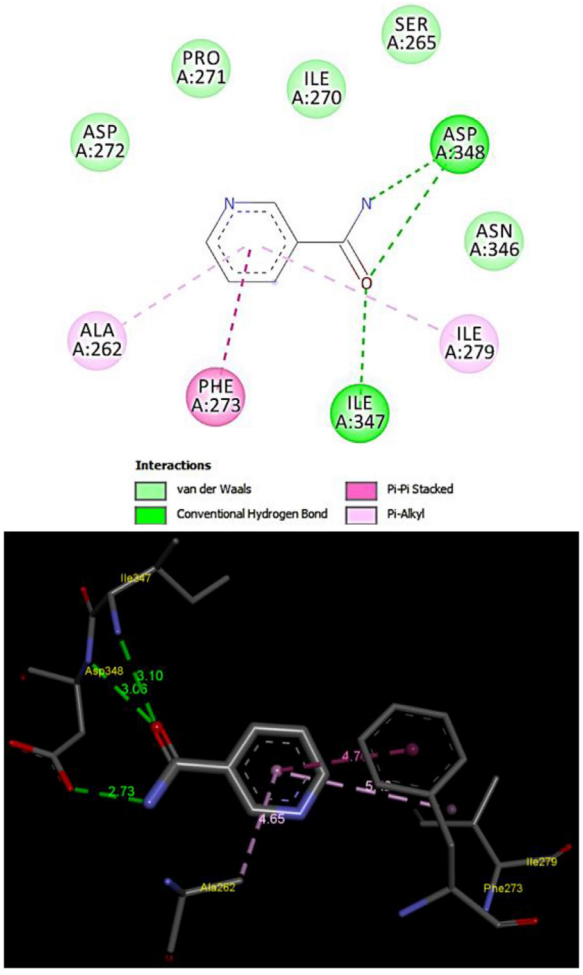
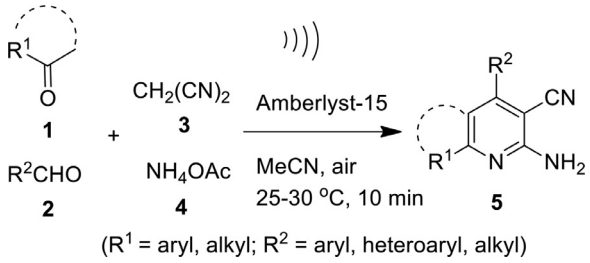


Fig. 6. Binding interactions and docked pose of nicotinamide at the catalytic site of SIRT1 (PDB: 4I5I).



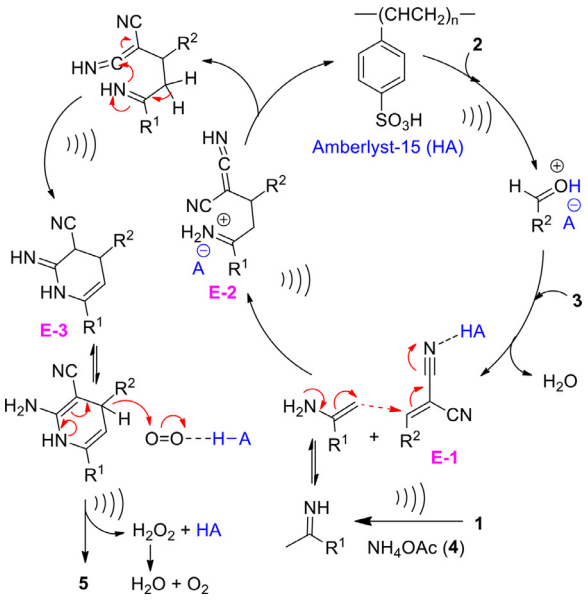
Scheme 1. Amberlyst-15 catalysed sonochemical synthesis of 2-amino-4,6-disubstituted nicotinonitrile derivatives.

Ravikumar Kapavarapu and Varadacharyulu Nallanchakravarthula was involved in the investigation and formal analysis using the software.

Naresh Kumar Katari and Manojit Pal were responsible for conceptualization, supervision and project administration of the entire work presented in the submitted manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



Scheme 2. Proposed reaction mechanism for the Amberlyst-15 catalysed sonochemical synthesis of 2-amino-4,6-disubstituted nicotinonitrile derivatives (**5**).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.130541.

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