An Efficient Green Multi-Component Reaction Strategy for the Synthesis of Highly Functionalised Pyridines and Evaluation of Their Antibacterial Activities

Lakkireddy Srinivasula Reddy,[a,b] Tirumalareddy Ram Reddy,[b] Reddy Bodireddy Mohan,[c] Avula Mahesh,[b] Yeramanchi Lingappa,[a] and Nallagondu Chinna Gangi Reddy*[,c]

[a] *Department of Chemistry, Sri Venkateswara University; Tirupati–517 502, Andhra Pradesh, India:* [b] *Dr. Reddy's Laboratories Limited; Bollaram Road, Miyapur, Hyderabad–500 049, Andhra Pradesh, India: and* [c] *Department of Chemistry, School of Physical Sciences, Yogi Vemana University; Kadapa–516 003, Andhra Pradesh, India.*
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An efficient green multi-component reaction (MCR) method has been developed for the synthesis of 2-amino-4-aryl/heteroaryl-6-(pyridin-2-ylthio)pyridine-3,5-dicarbonitrile(s) *via* a 3-component reaction of aryl aldehyde(s), malononitrile and 2-mercaptopyridine in the presence of K_2CO_3 under solvent free reaction conditions (SFRC) using grinding technique at room temperature in a single step. The advantages of the present protocol is operationally simple, environmentally benign, solvent-free reaction conditions (SFRC), simple work up, excellent isolated yields of desired products and viable method for large scale applications in pharmaceutical industry. Interestingly, the synthesized compounds showed moderate to excellent antibacterial activities against Gram-positive and Gram-negative bacterial strains.

Key words pyridine; solvent-free synthesis; green chemistry; multi-component reaction; grindstone chemistry; antibacterial activity

The pharmaceutical industry is increasingly under pressure to minimise its environmental impact.[1)] Demands for sustainable and environmental friendly organic syntheses[2,3)] are stimulating the search for alternatives to the use of organic solvents in synthetic organic chemistry. Performing reaction under solvent-free reaction conditions (SFRC) using grinding technique is an important and interesting alternative, but reaction pathways, as well as the products formed may be modified significantly.[4–11)] 2-Amino-3,5-dicarbonitrile-6-thiopyridines are among the most important *N*-heterocycles that exhibit miscellaneous pharmacological activities such as anti-prion,[12,13)] anti-hepatitis B,[14)] anticancer agents[15)] and some of these compounds are recognized as highly selective ligands for adenosine receptors,[16)] which are renowned as possible targets for the development of new drugs in the treatment of Parkinson's disease, asthma, kidney disease, epilepsy, and cancer.[17)] These utilities continue to drive the interest in the development of new synthetic routes for 2-amino-3,5-dicarbonitrile-6-thiopyridines which include a multi-component reactions (MCRs) under basic conditions[18–21)] or basic ionic liquid medium[22)] and also MCR using Lewis acid catalysts such as ($ZnCl_2$)[23)] and boric acid,[24)] which show improved results when compared to base-catalyzed reactions. Ultrasonic-assisted synthesis also reported in literature.[25–29)] However, this reported method is not accessible for large scale applications in pharmaceutical industry. Since grindstone chemistry[30)] is inexpensive and viable method for scale-up process when compared to Ultrasonic-assisted synthesis. Nevertheless, to the best of our knowledge, no reports on one-pot synthesis of 2-amino-4-aryl/heteroaryl-6-(pyridin-2-ylthio)pyridine-3,5-dicarbonitrile *via* MCR under SFRC using grindstone technology.

The present communication describes an one-pot three component synthesis of 2-amino-4-aryl/heteroaryl-6-(pyridin-2-ylthio)pyridine-3,5-dicarbonitrile (**4**) from aryl aldehyde (**1**), malononitrile (**2**) and 2-mercaptopyridine (**3**) in presence of K_2CO_3 under SFRC using grinding technique at room temperature as shown in Chart 1.

Mechanistically, the reaction seems to proceed (Chart 2) *via in situ* generation of 2-alkylidenemalononitrile *i.e.*, Knoevenagal adduct (**5**) from aryl aldehyde (**1**) and malononitrile (**2**) under basic conditions employed. Further, firstly, 1,2-addition between the thiol group of 2-mercaptopyridine and one of the nitrile group of another malononitrile and secondly, Michael

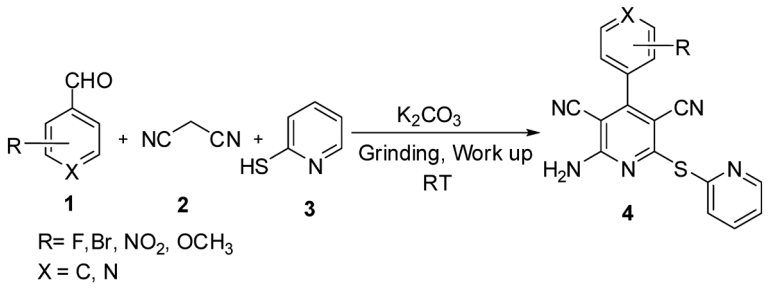


Chart 1. Green MCR Strategy for the Synthesis of 2-Amino-4-aryl/Heteroaryl-6-(pyridin-2-ylthio)pyridine-3,5-dicarbonitriles

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: ncgreddy@yogivemanauniversity.ac.in

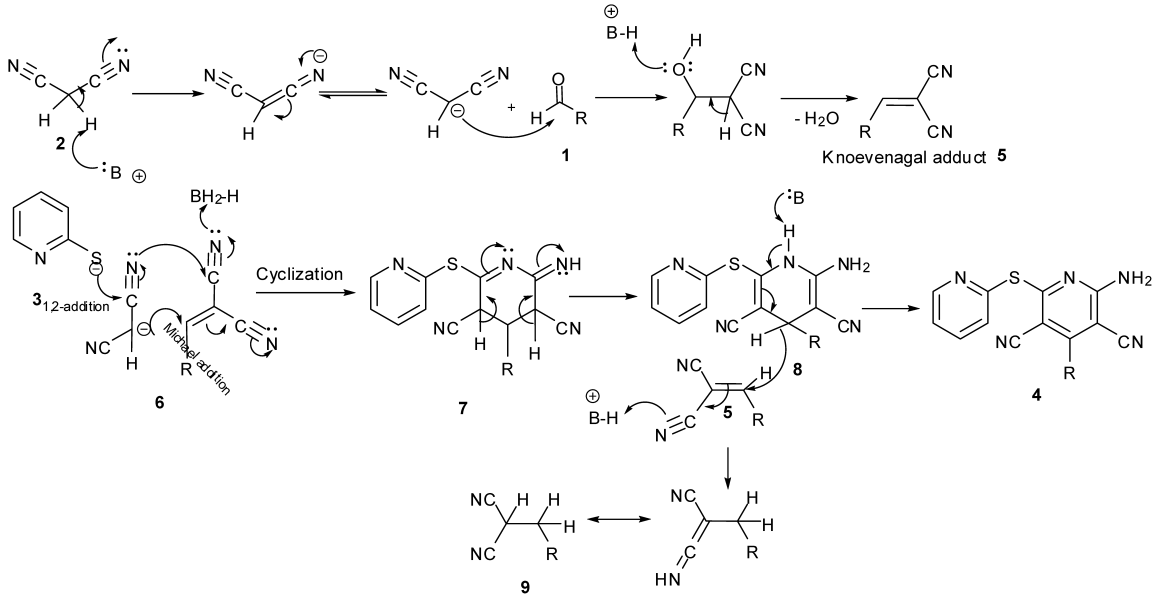


Chart 2. Plausible Mechanism[19] for the Formation of 2-Amino-4-aryl/Heteroaryl-6-(pyridin-2-ylthio)pyridine-3,5-dicarbonitrile

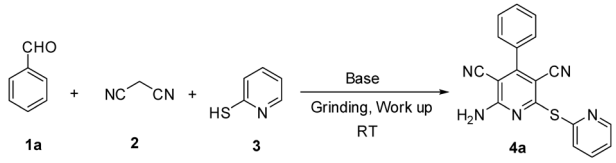
addition between carbanion of malononitrile and Knoevenagel adduct (**5**) followed by cyclization afforded the intermediate (**7**) which tautomerises to 1,4-dihydropyridine intermediate (**8**) and subsequent aromatization in the presence of base and Knoevenagel adduct **5** (an *in situ*-oxidizing agent) provided the desired product 2-amino-4-aryl/heteroaryl-6-(pyridin-2-ylthio)pyridine-3,5-dicarbonitrile(s) **4a**–**i** and compound **9**.

Results and Discussion

In our initial study, the reaction of benzaldehyde (**1a**), malononitrile (**2**) and 2-mercaptopyridine (**3**) was carried out in the presence of Na_2CO_3 at room temperature give rise to 2-amino-3,5-dicarbonitrile-4-phenyl-6-thio pyridine **4a**. It was isolated and yield is 42% in water and 63% under solvent-free reaction conditions (SFRC) (entry 1, Table 1). Then, we conducted the same reaction using K_2CO_3 and afforded 52% of yield in water and highest yield of 87% of product **4a** was obtained under SFRC at room temperature. The use of other bases such as *N*-methyl-2-pyrrolidone, L-proline, basic Al_2O_3 and Cs_2CO_3 were examined, but were found to be less effective as the expected product **4a** was isolated in 30–50% of yield (entries 3–6) in water and 40–70% of yield under SFRC. We also carried out the same reaction using tetra-*n*-butylammonium fluoride, KH_2PO_4, $BaCl_2$ both in water and under SFRC, but the obtained yield was not satisfactory (entries 7–9). From the above study we concluded that K_2CO_3 is the best option to get maximum yield of the desired product **4a** under SFRC using grinding technique at room temperature.

Further, the effect of temperature was examined under SFRC using K_2CO_3 (also with other bases stated in Table 1). In this connection, we raised the temperature of the reaction to 70–80°C and maintained at the same temperature for

Table 1. Optimization of Reaction Conditions for Synthesis of 2-Amino-4-phenyl-6-(pyridin-2-ylthio)pyridine-3,5-dicarbonitrile (**4a**) *via* MCR of **1a**, **2** and **3**[a]



1a 2 3 4a

Entry	Base	Time (min)		Yield[b] (%)	
		Solvent (H_2O)	SFRC[c]	Solvent (H_2O)	SFRC[c]
1	Na_2CO_3	150	60	42	63
2	K_2CO_3	120	30	52	87
3	NMP	210	65	48	68
4	L-Proline	250	70	35	57
5	Basic Al_2O_3	300	60	20	46
6	Cs_2CO_3	330	60	40	61
7	TBAF	250	55	46	70
8	$K_2H_2PO_4$	270	60	45	71
9	$BaCl_2$	400	70	30	52

a) Reaction conditions: Benzaldehyde (**1a**) (0.00188 mol), malononitrile (**2**) (0.00377 mol), 2-mercaptopyridine (**3**) (0.0015 mol), base (1 eq) and H_2O (10 vol) or SFRC at room temperature. *b*) Isolated yields of the product. *c*) Reaction carried out under stirring. *d*) Reaction carried out under solvent free reaction conditions (SFRC) using grinding technique.

Table 2. Effect of Base Load on Synthesis of 2-Amino-4-phenyl-6-(pyridin-2-ylthio)pyridine-3,5-dicarbonitrile (**4a**)[a]

Entry	K_2CO_3 (eq)	Time (min)	Yield[b] (%)
1	0.5	70	40
2	0.75	60	58
3	1	30	87
4	1.5	40	82
5	2.0	40	78

a) Reaction conditions: Benzaldehyde (**1a**) (0.00188 mol), malononitrile (**2**) (0.00377 mol), 2-mercaptopyridine (**3**) (0.0015 mol), K_2CO_3 at room temperature under SFRC. *b*) Isolated yields of product (**4a**).

30–70 min. The obtained yield of product **4a** was comparatively low (50–65%). Based on obtained results, we concluded that room temperature is optimum for maximum conversion and highest isolated yield of product **4a**.

Base concentration was optimized by varying its concentration from 0.5 to 2.0 eq (entries 1–5, Table 2). Increase in the product (**4a**) yield was observed on apply of 0.5 to 1.0 eq of base. Decrease in yield of the product **4a** was observed on increase of base concentration (entries 4–5). Hence, 1 eq of base was considered as an optimum base concentration (entry 3).

To test the generality and scope of this MCR a range of aryl aldehydes (**1a**–**i**) were employed and obtained results were presented in Table 3. Consistent with the results in the optimization studies, the reactions smoothly proceeded to afford 2-amino-4-aryl/heteroaryl-6-(pyridin-2-ylthio)pyridine-3,5-dicarbonitriles in good to excellent isolated yields. Various electron donating *e.g.* OMe, Br, OH (entries 2, 5, 6, 8, Table 3) or electron withdrawing groups *e.g.* F, NO_2 (entries 3, 4, 7) present on the aryl aldehydes were well tolerated. The reaction proceeded well irrespective of the substituents present on aryl aldehydes (entries 1 *vs.* 2–8). The use of heteroaromatic aldehyde was also successful and afforded the desired product **4i** in good yield (entry 9).

Antibacterial Activity Studies The primary antimicrobial activity screen was carried out by agar disc-diffusion method.[31–34] All the synthesized compounds were tested by the disk diffusion method against Gram-positive bacterial strains: *Micrococcus luteus* (MTCC 106) and *Staphylococcus aureus* (MTCC 737) and Gram-negative bacterial strains: *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 109). Chloramphenicol was used as positive control and dimethyl sulfoxide (DMSO) was used as negative control. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. The minimum inhibitory concentration (MIC) for the most active compounds **4c**, **4d**, **4g** and **4i** against the same microorganisms used in the preliminary screening was carried out using microdilution susceptibility method[32] and presented in Table 4.

The results of preliminary antibacterial testing of compounds **4a**–**i** are shown in Table S1 (see supplementary information). The results revealed that, compounds **4c**, **4d**, **4g** and **4i** displayed good activity against Gram-positive bacteria (inhibitory zone >25 mm) and excellent activity against Gram (−Ve) bacteria (inhibitory zone >27 mm). Compound **4e** displayed low to moderate activity towards Gram (+Ve) bacteria (18–21 mm) and moderate activity (20–23 mm) towards Gram (−Ve) bacteria. Compounds **4a**, **4b** and **4h** exhibited least activity against both bacteria.

On the other hand, the results of preliminary antibacterial testing of compounds **4a**–**i** as shown in Table 4 revealed that, compounds **4d** and **4i** exhibited good activity against Gram (+Ve) bacteria (inhibitory zone >27 mm) and excellent activity against Gram (−Ve) bacteria (inhibitory zone >31 mm). Compounds **4c**, **4e** and **4g** showed moderate activity towards Gram (+Ve) bacteria (19–27 mm) and moderate to good activity (23–29 mm) towards Gram (−Ve) bacteria.

The MIC values were determined as the lowest concentration that completely inhibited visible growth of the microorganisms as presented in Table S1. The structure–antimicrobial activity relationship of the synthesized compounds revealed that presence of electron withdrawing groups (F, Br, NO_2 on 4′-aryl substituent or presence of 4′-heterocycle of pyridine core displayed high activity. The compounds having electron donating groups (OH, OMe) on 4′-aryl substituent of pyridine core or without substitution exhibited low activity. Excellent activity was attained with the compounds (**4c**–**d**) having –F (fluorine) substituent on the 4′-aryl substituent of pyridine core. The maximum activity was observed with compound (**4i**) having 4′-heterocycle substituent of pyridine core.

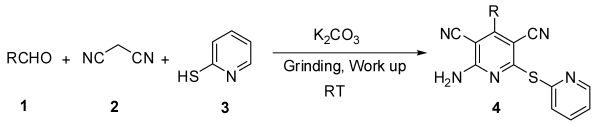
Conclusion

In summary, we demonstrated an efficient Green MCR strategy for the synthesis of highly functionalized pyridines (**4a**–**i**) of potential medicinal interest *via* a three component reaction under SFRC by adopting Grindstone chemistry in presence of K_2CO_3 in a single step. The advantages of the grindstone technique include high yields, environmentally benign procedure, shorter reaction time, simpler workup procedure and less energy consumption. The methodology is also useful in constructing a diversity based library of molecules related to Pyridine framework. Among the synthetic analogues, compounds **4d** and **4i** showed excellent inhibition against multidrug resistant Gram-negative bacteria, *E. coli* and *K. pneumoniae*, nearly equivalent to that of reference drug Chloramphenicol. We have discerned that the inhibition rate was directly proportional either to strength of electron withdrawing groups on 4-aryl substituent or presence 4-heteroaryl group of 2-amino-4-aryl-6-(pyridin-2-ylthio)pyridine-3,5-dicarbonitrile, **4c**–**e**, **4g** and **4i**.

Experimental

Melting points were determined using Buchi (B-540) melting point analyzer (Postfach, Flawil, Switzerland) and are uncorrected. NMR spectra were recorded on Varian 400 MHz (Varian, Inc., NMR Systems, Palo Alto, CA, U.S.A.). Chemical shifts were expressed in parts per million (ppm), coupling constants were expressed in Hertz (Hz). Splitting patterns describe apparent multiplicities and were designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). High Resolution Mass Spectra were recorded on a LCT-Premier XE mass spectrometer (Waters, Milford, MA, U.S.A.)-single quadrupole system equipped with electrospray ionization (ESI) source. Thin-layer chromatography was performed on 0.25 mm Merck silica gel plates and visualized with UV light. All chemicals and solvents are purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.) and Merck (Mumbai, India) and are used without further purification.

Table 3. Synthesis of 2-Amino-4-aryl/Heteroaryl-6-(pyridin-2-ylthio)pyridine-3,5-dicarbonitrile (**4a–i**)[a)]



1 2 3 4

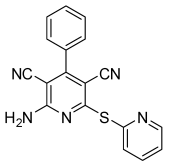
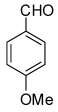
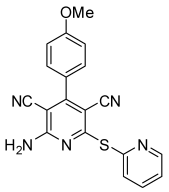
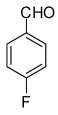
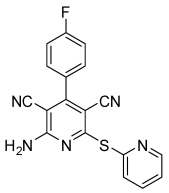
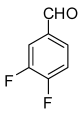
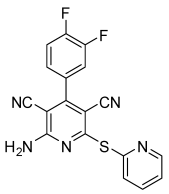
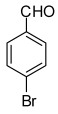
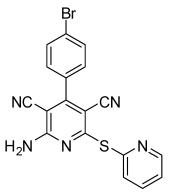
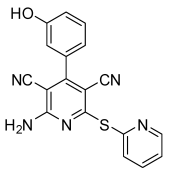
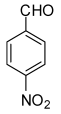
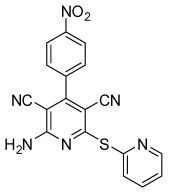
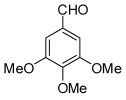
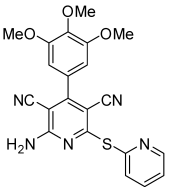
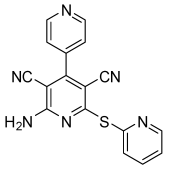
Entry	Aryl aldehyde (R) (**1**)	Time (min)	Product(s) (**4**)	Yield[b)] (%)
1	 **1a**	30	 **4a**	87
2	 **1b**	20	 **4b**	82
3	 **1c**	25	 **4c**	91
4	 **1d**	30	 **4d**	90
5	 **1e**	20	 **4e**	88
6	 **1f**	28	 **4f**	83

Table 3. Continued

Entry	Aryl aldehyde (R) (**1**)	Time (min)	Product(s) (**4**)	Yield[b] (%)
7	 **1g**	35	 **4g**	85
8	 **1h**	15	 **4h**	92
9	 **1i**	20	 **4i**	90

a) Reaction conditions: Aryl aldehyde(s) (**1a–i**) (0.00188 mol), malononitrile (**2**) (0.00377 mol), 2-mercaptopyridine (**3**) (0.0015 mol), K_2CO_3 (1 eq) at room temperature under SFRC. *b*) Isolated yields of product(s) (**4a–i**).

Table 4. Minimum Inhibitory Concentration (MIC), μg/mL of **4**

Compound	Minimum inhibitory concentration (μg/mL)			
	Gram-positive bacteria		Gram-negative bacteria	
	M. luteus	*S. aureus*	*E. coli*	*K. pneumoniae*
4c	100	100	100	100
4d	100	75	100	200
4g	50	100	100	100
4i	25	50	25	50
Chloramphenicol	6.5	6.5	6.25	6.0

General Experimental Procedure for Synthesis of Compounds 4a–i A mixture of benzaldehyde (**1a**) (0.00188 mol, 0.2 g), malononitrile (**2**) (0.00377 mol, 0.249 g), K_2CO_3 (0.00188 mol, 0.138 g) and 2-mercaptopyridine (**3**) (0.00150 mol, 0.167 g) were thoroughly ground with a pestle in a mortar at room temperature in an open atmosphere until the mixture turned into a thick mass. The progress of the reaction was monitored by TLC. The mixture continued to be ground occasionally until completion of the reaction. After completion of the reaction as from TLC, water (100 mL) was added to the crude mass and product was extracted using ethyl acetate (3×50 mL). The organic layer was collected, dried over anhydrous Na_2SO_4 and the solvent was removed under vacuum. Pure product **4a** was obtained from crude mass after recrystallisation from a mixture of EA–*n*-hexane (1 : 1 ratio, 10 vol) and the crude 2-benzylmalononitrile (**9a**) was isolated from the filtrate and purified by column chromatography using a mixture of EA–*n*-hexane (1 : 99 ratio). The obtained by-product 2-benzylmalononitrile (**9a**) was confirmed by ^{1}H-NMR and GC-MS. The same procedure for experimental and purification was followed for the preparation of all other compounds (**4b–i**). The synthesised compounds (**4a–i**) gave satisfactory spectroscopic data in accordance with their proposed structures.

2-Amino-4-phenyl-6-(pyridine-2-ylthio)pyridine-3,5-dicarbonitrile (**4a**): Off-white solid Yield=87%; mp 224–227°C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.57 (d, 1H, J=3.6 Hz, arom H), 7.98 (br, 2H, –NH_2), 7.87 (t, 1H, J=8.0 Hz, arom H), 7.8 (d, 1H, J=8.0 Hz, arom H), 7.58 (s, 5H, arom H), 7.43 (t, 1H, J=6.0 Hz, arom H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 164.2, 159.8, 158.9, 152.4, 150.2, 137.8, 133.9, 130.5, 129.6, 129.4, 128.8 (2C), 128.6, 128.4 (2C), 123.5, 95.6, 88.0; high resolution (HR)-MS (ESI): m/z $(M+H)^+$ Calcd for $C_{18}H_{12}N_5S$: 330.0813, Found 330.0808.

2-Benzylmalononitrile (**9a**): White solid; mp 88–90°C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.43–7.38 (m, 2H, arom H), 7.36–7.30 (m, 2H, arom H), 3.90 (t, J=6.8 Hz, 1H), 3.27 (d,

J=6.8 Hz, 2H); GC-MS: m/z $(M)^+$ 156.

2-Amino-4-(4-methoxyphenyl)-6-(pyridine-2-ylthio)-pyridine-3,5-dicarbonitrile (**4b**): Off-white solid; Yield=82%; mp 250–253°C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.57 (d, 1H, J=4.0 Hz, arom H), 7.93 (br, 2H, –NH_2), 7.86 (td, 1H, J=8.0, 2.0 Hz, arom H), 7.80 (d, 1H, J=8.0 Hz, arom H), 7.53 (d, 2H, J=8.8 Hz, arom H), 7.42 (q, 1H, J=4.8 Hz, arom H), 7.13 (d, 2H, J=8.0 Hz, arom H), 3.85 (s, 3H, –OCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 164.2, 160.9, 159.9, 158.5, 152.5. 150.2, 137.8, 130.3 (2C), 128.5, 125.7, 123.4, 115.5, 115.2, 114.1 (2C), 95.7, 87.9, 55.4; HR-MS (ESI): m/z $(M+H)^+$ Calcd for $C_{19}H_{14}N_3OS$: 360.0919, Found: 360.0906.

2-Amino-4-(4-fluorophenyl)-6-(pyridine-2-ylthio)-pyridine-3,5-dicarbonitrile (**4c**): Off-white solid; Yield= 91%; mp 246–249°C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.57 (d, 1H, J=4.0 Hz, arom H), 8.0 (br, 2H, –NH_2), 7.87 (td, 1H, J=8.0, 2.0 Hz, arom H), 7.80 (d, 1H, J=8.0 Hz, arom H), 7.67–7.64 (m, 2H, arom H), 7.46–7.41 (m, 3H, arom H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 164.2, 159.7, 157.9, 152.4, 150.2, 137.8 (2C), 131.2, 131.1, 128.6 (2C), 123.5, 116.0, 115.8, 115.2, 114.8, 95.7, 88.1; HR-MS (ESI): m/z $(M+H)^+$ Calcd for $C_{18}H_{11}N_5FS$: 348.0719, Found: 348.0704.

2-Amino-4-(3,4-difluorophenyl)-6-(pyridine-2-ylthio)-pyridine-3,5-dicarbonitrile (**4d**): Off-white solid; Yield=90%; mp 251–254°C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.58 (d, 1H, J=4.8 Hz, arom H), 8.06 (br, 2H, –NH_2), 7.88 (t d, 2H, J=8.0, 2.0 Hz, arom H), 7.80 (d, 1H, J=7.6 Hz, arom H), 7.73–7.6 (m, 1H, arom H), 7.5–7.43 (m, 2H, arom H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 164.2, 159.6, 156.6, 152.2 (2C), 150.2, 137.8, 128.7, 126.2, 123.6, 118.49, 118.43, 118.3, 118.2, 115.0, 114.6, 95.5, 88.2; HR-MS (ESI): m/z $(M+H)^+$ Calcd for $C_{18}H_{10}N_5F_2S$: 366.0625, Found: 366.0606.

2-Amino-4-(4-bromophenyl)-6-(pyridine-2-ylthio)-pyridine-3,5-dicarbonitrile (**4e**): Off-white solid; Yield=88%; mp 260–263°C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.57 (d, 1H, J=4.0 Hz, arom H), 8.0 (br, 2H, –NH_2), 7.87 (td, 2H, J=8.0, 1.6 Hz, arom H), 7.81 (d,t, 2H, J=8.4, 6.0 Hz, arom H), 7.54 (d, 2H, J=8.4 Hz, arom H), 7.44–7.41 (m, 1H, arom H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 164.2, 159.7, 157.7, 152.3, 150.2, 137.8, 133.1, 131.8 (2C), 130.6 (2C), 128.6, 124.2, 123.5, 115.1, 114.8, 95.4, 87.9; HR-MS (ESI): m/z $(M+H)^+$ Calcd for $C_{18}H_{11}N_5SBr$: 407.9919, Found: 407.9904, other isotopic peaks found: 409.8890, 410. 9925

2-Amino-4-(3-hydroxyphenyl)-6-(pyridine-2-ylthio)-pyridine-3,5-dicarbonitrile (**4f**): Off-white solid; Yield=83%; mp 222–226°C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.89 (s, 1H, –OH), 8.56 (d, 1H, J=3.6 Hz, arom H), 7.95 (br, 2H, –NH_2), 7.87 (td, 1H, J=8.0, 2.0 Hz, arom H), 7.80 (d, 1H, J=8.0 Hz, arom H), 7.43–7.35 (m, 2H, arom H), 6.97–6.89 (m, 3H, arom H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 164.1, 159.8, 158.8, 157.3, 152.5, 150.1, 137.8, 135.0, 130.0, 128.5, 123.4, 118.9, 117.3, 115.2, 115.0, 114.8, 95.5, 87.9; HR-MS (ESI): m/z $(M+H)^+$ Calcd for $C_{18}H_{12}N_5OS$: 346.0763, Found: 346.0746.

2-Amino-4-(4-nitrophenyl)-6-(pyridine-2-ylthio)-pyridine-3,5-dicarbonitrile (**4g**): Off-white solid; Yield=85%; mp 245–248°C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.58 (d, 1H, J=4.0 Hz, arom H), 8.44 (d, 2H, J=8.8 Hz, arom H), 8.11 (br, 2H, –NH_2), 7.90 (d, 2H, J=8.4 Hz, arom H), 7.87 (d, 1H, J=2.0 Hz, arom H), 7.81 (d, 1H, J=8.0 Hz, arom H), 7.44 (dd, 1H, J=6.8, 4.8 Hz, arom H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 164.4, 159.6, 156.9, 152.2, 150.2, 148.6, 140.1, 137.9, 130.3 (2C), 128.7, 123.9 (2C), 123.6, 114.9, 114.5, 95.1, 87.8; HR-MS (ESI): m/z $(M+H)^+$ Calcd for $C_{18}H_{11}N_6O_2S$: 375.0664, Found: 375.0657.

2-Amino-4-(3,4,5-trimethoxyphenyl)-6-(pyridine-2-ylthio)-pyridine-3,5-dicarbonitrile (**4h**): Off-white solid; Yield=92%; mp 265–268°C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.57 (dd, 1H, J=4.8, 1.2 Hz, arom H), 7.95 (br, 2H, –NH_2), 7.87 (td, 1H, J=8.0, 1.6 Hz, arom H), 7.79 (d, 1H, J=8.0 Hz, arom H), 7.43 (td, 1H, J=5.6, 2.4 Hz, arom H), 6.94 (s, 2H, arom H), 3.82 (s, 6H, –2OCH_3), 3.76 (s, 3H, –OCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 164.1, 159.7, 158.5, 152.7, 152.4, 150.1, 138.8, 137.7, 128.8, 128.6 (2C), 123.4, 115.4, 115.0, 106.4 (2C), 95.5, 87.9, 60.1, 56.1 (3C), HR-MS (ESI): m/z $(M+H)^+$ Calcd for $C_{21}H_{18}N_5O_3S$: 420.1130, Found: 420.1117.

2-Amino-4-(pyridine-2-ylthio)-4-(pyridine-4-ylthio)-pyridine-3,5-dicarbonitrile (**4i**): Off-white solid; Yield=90%; mp 233–235°C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.82 (dd, 2H, J=4.4, 1.6 Hz, arom H), 8.58 (dd, 1H, J=4.8, 1.2 Hz, arom H), 8.1 (br, 2H, –NH_2), 7.88 (td, 1H, J=7.6, 2.0 Hz, arom H), 7.81 (d, 1H, J=8.4 Hz, arom H), 7.61 (d, 2H, J=6.4 Hz, arom H), 7.45–7.42 (m, 1H, arom H).; ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 164.3, 159.5, 156.3, 152.1, 150.2 (3C), 141.6, 137.8, 128.6, 123.5, 122.8 (2C), 114.8, 114.4, 94.8, 87.5.; HR-MS (ESI): m/z $(M+H)^+$ Calcd for $C_{17}H_{11}N_6S$: 331.0766, Found: 331.0754.

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