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Synthesis of 2, 6-Diaryl-4-5-Secondary aminonicotinonitriles as potent antimicrobial agents

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Abstract: A series of 2, 6-Diaryl-4-5-Secondary aminonicotinonitriles derivatives were synthesized by the reaction of 2H-pyran-2-one (**I)** and the N-aryl amidine (**II**) using KOH as catalyst in DMF at room temperature. All synthesized compounds (**IIIa-i**) evaluated for antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Proteus vulgaris, Aspergillus niger* and, *Candida albicans* strains *most* of the compound shows potent activity.

Keywords: 2-H-pyran-2-one, N-aryl amidine, aminonicotinonitrile and Antimicrobial activity.

1. Introduction

Pyridine and its derivatives are one of the most important class of heterocycles found in many natural products like vitamin-B12 (Niacin and Pyridoxal), alkaloids (Nicotine) and it act as key precursor for synthesis of various medicines and agrochemicals. In recent past, after considering the novel insecticides belonging to the group, neonicotinoids[1-2] like imidacloprid and nicotin novel derivatives of pyridine have been developed and used as antibacterial, antifungal[3], insecticidal[4] and pesticidal[5-6] agents. It was also found that 2-(p-aminobenzamido) pyridines exhibit a powerful inhibiting effect on gastric ulcers in rats [7]. The pyridines 2,2'-bi- and 2,2', 2''-ter-pyridine were used as metal chelating legends with various substituents [8]. Similarly, pyridine derivatives are of growing relevance in material science and supramolecular chemistry [9]. Therefore, there is a continuous interest to develop the new synthetic methods for pyridines and their derivatives. Classical routes to pyridine synthesis discovered by Hantzsch [10], Chichibabin [11], Petrenko-Kritschenko and Zoneff [12], Krohnke [13], and Guareschi-Thorpe [14] condensation reactions. The condensation of 1,5-diketone with ammonia followed by nitric acid oxidation is a common approach for the synthesis of pyridines [15]. The reaction of dienamine and ketone in the presence of Vilsmeier type 1-substituted-1,2,3-benzotriazole reagent results in the formation of nicotinonitriles [16]. The construction of unsymmetrically substituted pyridines was achieved by the reaction of 1,3-dicarbonyl compounds and 3-aminoenones or nitriles [17]. Saikai et al. reported indium trichloride catalyzed synthesis of tetrasubstituted pyridines [18]. Penieres et al. have synthesized pyridine by using microwave irradiated Hantzsch reaction [19]. Combinatorial approach also has been used for the synthesis of pyridine derivatives [20]. 2,4,6-trisubstituted pyridine derivatives were prepared from aroylketene dithioacetal by Potts et al [21]. Recently, Ram and coworkers [22] have described the use of 2H-pyran-2-one for the synthesis of substituted pyridines. The chemical structures of the synthesized compounds were confirmed by means of IR, 1H-NMR, Mass and C, H, N elemental analysis. The synthesized compounds were screened for antibacterial activity against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis*, *Pseudomonas aeruginosa* and fungi *Aspergillus niger* and *Candida albicans.*

2. Results and Discussion

2.1 Chemistry

We investigated that compound **III a-i** in moderate yield can be constructed from 2H-pyran-2-one **I** and the N-aryl amidine **II** via a ring transformation reaction using KOH in DMF at room temperature (Scheme1). Pyridine **III a-i** isolated in this study could arise by nucleophilic attack of amidine N-1 at C-6 position of 2H-pyran-2-one. The intermediate 4 formed is unstable and it undergoes cyclization with a retro [2 + 2] process to yield **III a-i** with the loss of carbon dioxide and yields 2,6-Diaryl-4-5-Secondary aminonicotinonitriles as target compounds.

2.2 Microbiology

The antimicrobial activities of these compounds were evaluated by minimum inhibitory concentration (MIC) against Gram-positive and Gram-negative test bacteria *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 1650), *Pseudomonas aeruginosa*, (MTCC 1688), and fungi *Aspergillus niger* (MTCC 1789) and *Candida albicans* (MTCC 227) *in vitro.* Streptomycin and nystatin as reference drug, by agar diffusion method, [23-26] the

results of *in vitro* antibacterial activities and MIC's of compound **III a-i** against various bacterial strains are summarized in **Table 2**, compound **III a, III c,** shows excellent activity against all bacteria with showing more than 12 mm zone of inhibition at 25 µg/mL MIC value. Compound **III f** is found to be potent activity against *Bacillus subtilis* and *Escherichia coli* even at very low concentration where as compound **III i** also point out excellent activity against *Pseudomonas aeruginosa*, with showing above 15 mm zone of inhibition even at 25 MIC value in competition with standard drug, where as compound **III f** is found to be almost equally potent active against *Escherichia coli* in comparison with streptomycin even at lowest MIC (25 ug /ml). Compound **III b**, **III d**, **III e**, and **III h** are also found good active but at double concentration. Compound **III a** shows considerable activity against *Aspergillus niger* where as compound **III c** and **III f** shows good activity against both fungal species. Compound **III i** found to most potent active towards *Aspergillus niger* and *Candida albicans* with showing more than 12 mm zone of inhibition at 25 µg/ mL MIC value in comparison with standard drug. Likewise remaining compound **III b**, **III d**, **III e**, **III g** and, **III h** are also found active but above 25 µg/ mL.

3. Experimental

3.1. Chemistry

All chemicals and solvents used were laboratory grade and directly used. Melting points were determined by open capillary method and are uncorrected. ^{1}H NMR spectra were recorded (in DMSO-d_6, □ ppm) on AVANCE-300 MHz spectrometer using TMS as an internal standard (s = singlet, d = doublet, t = triplet, m = multiplates and br = brod). Coupling constant (*J*) are given in (Hz). IR spectra were recorded (in KBr pallets) on SCHIMADZU spectrophotometer. Mass spectra were recorded on EI-SHIMDZU-GC-MS spectrometer. Elemental analyses were performed on a Perkins-Elmer C, H, N, elemental analyzer. All reactions and purity of isolated product was monitored by using thin layer chromatography (TLC) using 0.2 mm silica gel plates 60 F_{254} (MERCK) and mobile phase petroleum ether and ethyl acetate (80:20). Reaction components were visualized in UV (255 and 365 nm) and iodine chamber.

3.2. General procedure for Preparation of 2,6-Diaryl-4-secondary aminonicotinonitriles (III a-i)

The mixture of 2H-pyran-2-one-3-carbonitrile (1.0 mmol), N-phenyl benzamidine (1.0 mmol) and powdered KOH (2.0 mmol) in 5 mL DMF was stirred for 5–6 h at room temperature. The reaction was monitored by TLC. After completion of the reaction, excess DMF was removed under reduced pressure. Then the residue was poured into crushed ice with vigorous stirring. The aqueous solution was neutralized with 1 N, HCl, the precipitate obtained was filtered, and the obtained residue was purified by column chromatography on silica gel 60–120 by eluting 5:95 ethyl acetate: hexane as mobile phase.

Spectral data of selected compounds

IIIa) *2,6-Diphenyl-4-(piperidin-1-yl)pyridine-3-carbonitrile :* White solid, IR (KBr): 2209 (CN), 1568 (CN) cm^{-1}. 1H NMR (300 MHz, $CDCl_3$, 25° C) □ 1.73 (d, J =5.7 Hz,2H, CH_2), 1.83 (d, J = 3.3 Hz, 4H, $2CH_2$), 3.54 (t, J = 9.9 Hz, 4H, $2CH_2N$), 6.91 (s, 1H, CH), 7.5 (m, 5H, ArH), 7.84 (d, J = 4.7 Hz, 2H, ArH), 8.05 (t, J = 9.5 Hz, 3H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) □ =24.2, 26.1, 52.1, 96.4, 106.7, 1187, 124.4, 125.3, 127.6, 128.5, 128.9, 129.6, 130.1, 138.9, 159.8, 162.9, 164.2. MS (ESI, 70 eV) m/z (%) = 340 (100) [M+], 341(27) [$(M+H)^+$]. Anal. Calcd. for $C_{23}H_{21}N_3$: C, 81.38; H, 6.24; N, 12.38. Found: C, 81.42; H, 6.20; N, 12.38.

IIIc) 6-(4-Bromophenyl)-2-phenyl-4-(piperidin-1-yl)pyridine-3- carbonitrile: White solid, IR (KBr): 2212 (CN), 1568 (CN) cm^{-1}. 1H NMR (300 MHz, $CDCl_3$, 25°C) □ = 1.72 (d, 2H, CH_2), 1.8 (d, J = 5 Hz, 4H, $2CH_2$), 3.54 (t, J = 10.6 Hz, 4H, $2CH_2N$), 7.12 (s, 1H, CH), 7.51 (m, 3H, ArH), 7.59 (s, 1H, ArH), 7.62 (s, 1H, ArH), 7.93 (m, 4H, ArH). 13C NMR (75 MHz, CDCl3) □ =24.2, 26.1, 52.0, 96.8, 106.4, 118.6, 124.7, 128.6, 129.2, 129.6, 130.1, 132.1, 137.7, 138.7, 158.5, 162.9, 163.9. MS (ESI, 70 eV) m/z (%) = 418 (100) [M+], 420 (92) [(M+2H)+]. Anal. Calcd. for $C_{23}H_{20}BrN_3$: C, 66.04; H, 4.82; N, 10.04. Found: C, 66.01; H, 4.77; N, 10.08.

IIIf) *6-(4-Chlorophenyl)-4-(morpholin-4-yl)-2-phenylpyridine-3-carbonitrile:* White solid, IR (KBr): 2216 (CN), 1583 (C=N) cm^{-1}. 1H NMR (300 MHz,$_3$, 25°C) d =3.54 (q, J = 14.1 Hz, 4H, $2CH_2N$), 3.94 (q, J = 13.9 Hz, 4H, $2CH_2O$), 7.14 (s, 1H, CH), 7.51 (m, 5H, ArH), 7.89 (m, 2H, ArH), 8.00 (s, 1H, ArH), 8.04 (s, 1H, ArH). 13C NMR (75 MHz, $CDCl_3$) d = 50.9, 66.8, 97.2, 106.3, 118.2, 128.6, 128.9, 129.3, 129.5, 130.3, 136.7, 136.9, 138.3, 159.0, 162.7, 164.2. MS (ESI, 70 eV) m/z (%): 376 (100) [M+], 378 (33) [(M+2H)+]. Anal. Calcd. for $C_{22}H_{18}ClN_3O$: C, 70.30; H, 4.83; N, 11.18. Found: C, 70.34; H, 4.94; N, 11.23.

IIIh) *6-(4-Chlorophenyl)-2-phenyl-4-(pyrrolidin-1-yl)pyridine-3-carbonitrile:* White solid, IR (KBr): 2199 (CN), 1590 (C=N) cm^{-1}. 1H NMR (300 MHz, CDCl3, 25° C) d 2.09 (m, 4H, $2CH_2$), 3.81 (t, J = 13.2 Hz, 4H, $2CH_2N$), 6.85 (s, 1H, CH),

7.48 (m, 5H, ArH), 7.86 (q, J = 9.6 Hz, 2H, ArH), 7.98 (d, J = 8.66 Hz, 2H, ArH). 13C NMR (75 MHz, $CDCl_3$) □ =25.9, 50.5, 96.1, 106.5, 118.6, 128.4, 128.6, 129.0, 129.6, 129.8, 135.9, 137.5, 139.3, 158.6, 162.8, 163.1. MS (ESI, 70 eV) m/z (%) = 360 (100) [M+], 362 (32) [$(M+2H)^+$]. Anal. Calcd. for $C_{22}H_{18}ClN_3$: C, 73.43; H, 5.04; N, 11.68. Found: C,73.42; H, 5.04; N, 11.03.

3.3. Determination of Antimicrobial Activity

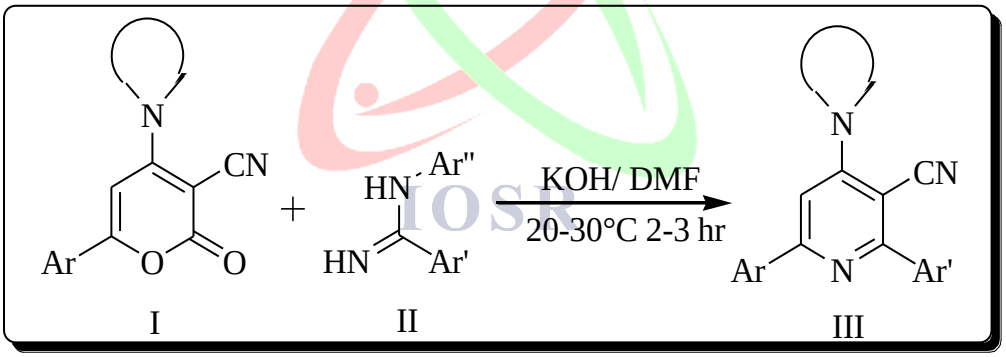
3.3.1. Antibacterial activity

The antibacterial activities of the synthesized compounds **III a-i** were determined by agar diffusion method as recommended by the National Committee for Clinical Laboratory Standards, (NCCLS) [23-25], against selected Gram-positive bacteria viz. *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 96) and Gram-negative bacteria viz. *Pseudomonas aeruginosa* (MTCC 1688), and *Escherichia coli* (MTCC 1650) strains by the agar well diffusion method. Briefly, 0.1 mL of overnight grown respective bacterial culture was spreaded over the nutrient agar plates. The wells of 6 mm diameter were prepared on the nutrient agar plates and filled with diluted test compounds separately. For comparison, DMSO and antibiotic Streptomycin were used as a solvent control and as reference antibacterial agent, respectively. Inoculated plates were then incubated at 37°C for 24 h and the resulting zones of inhibition (in mm) were measured. The minimum inhibitory concentrations at which no growth was observed was taken as the MIC value.

3.3.2. Antifungal activity

The compounds were screened for their antifungal activity on the fungal strains *Aspergillus niger* (MTCC 1789) and *Candida albicans* (MTCC 227). Fungal suspension (0.1 mL) was spread on Sabourauds agar plates. The wells of 6 mm diameter were prepared on the inoculated plates and filled with diluted test compounds separately. For comparison, DMSO and antibiotic Nystatin were used as solvent control and reference antifungal agent, respectively. Inoculated plates were then incubated at 30°C for 2-3 days and the resulting zones of inhibition (in mm) were measured. The minimum inhibitory concentrations at which no fungal growth observed was recorded as the MIC value.

4. Figuers and Tables



Sheme-1

Table: 1 Preparation of 2, 6-Diaryl-4-secondary aminonicotinonitriles 3 from 2H-pyran-2-one 1 and N-aryl amidine 2b

Entry	Ar	HN◯	Ar'	Ar''	Yield (%)	M. P. (°C)
III a	C6H5	Piperidine	C6H5	C6H5	46	160-162
III b	C6H5	Piperidine	C6H5	4-F-C6H4	48	173-174
III c	4-Br-C6H4	Piperidine	C6H5	C6H5	47	163-164
III d	4-Br-C6H4	Piperidine	C6H5	4-F-C6H4	43	197-199
III e	4-Br-C6H4	Morpholine	C6H5	4-F-C6H4	42	172-174
III f	4-Cl -C6H4	Morpholine	C6H5	C6H5	42	172-174
III g	4-Cl-C6H4	Morpholine	C6H5	4-F-C6H4	39	197-199
III h	4-Cl-C6H4	Pyrolidine	C6H5	C6H5	35	156-158
III i	4-Cl-C6H4	Pyrolidine	C6H5	4-Br -C6H4	38	137-138

Table:2 Antibacterial Activity of synthesized Compounds (III a-j)

Comp. No.	MIC in µg/mL (zone of inhibition, mm)					
	Antibacterial activity				Antifungal activity	
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. niger*	*C. albicans*
IIIa	25 (14)	25 (12)	25 (15)	25 (12)	25 (10)	25 (14)
IIIb	25 (6)	25 (7)	50 (8)	25 (10)	25 (10)	25 (10)
IIIc	25 (15)	25 (13)	25 (16)	25 (15)	25 (12)	25 (14)
IIId	50 (12)	50 (10)	25 (14)	25 (13)	50 (14)	50 (12)
IIIe	25 (10)	25 (10)	50 (14)	50 (12)	50 (10)	50 (10)
IIIf	25 (15)	50 (12)	25 (17)	50 (14)	25 (10)	25 (10)
IIIg	25 (10)	100 (10)	25 (12)	25 (12)	50 (10)	50 (10)
IIIh	50 (10)	50 (12)	25 (13)	25 (12)	25 (12)	25 (12)
IIIi	25 (12)	25 (14)	25 (13)	25 (15)	25 (18)	25 (17)
Streptomycin	25 (18)	25 (16)	25 (20)	25 (18)	ND	ND
Nystatin	ND	ND	ND	ND	6.25 (16)	25 (18)

5. Conclusion:

In summary, this work provides mild and efficient methodology for the synthesis of tetra substituted aminonicotinonitrile. Compound **III a, III c, III i** found to be potent antibacterial agents against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* respectively, where as compound **III c, III f** and **III i** shows predominant activity against *Aspergillus niger* and *Candida albicans* fungal species.

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