Synthesis and antimicrobial screening 6-Aryl-2-(Amino/methoxy)-4-[(4'-difluoro methoxy) (3'-hydroxy) phenyl] nicotinonitrile

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ABSTRACT

A series of an easy and efficient route for the synthesis of some new 2-amino-3-cyanopyridine (4a-4i) and 2-methoxy-3-cyanopyridine (5a-5i) derivatives were synthesized through the reaction of various chalcone (3a-3i) with malononitrile in presence of ammonium acetate and sodium methoxide, respectively. The constitutions of the all synthesized compounds have been established by the IR, ¹H-NMR, Mass spectral data and Elemental analysis. Furthermore, all the synthesized products were screened for their antimicrobial activity.

1. INTRODUCTION

Pyridine functionalities have been widely studied [1,2] and widely used [3-6] but still generate much interest due to their wide range of application in medicinal chemistry [7-11]. The naturally occurring B6-vitamins pyridoxine, pyridoxal, pyridoxamine and codecarboxylase contain a pyridine nucleus [12]. Pyridine derivatives have been used as herbicides [13], for enrichment of cereals [14], for regulation of arterial pressure [15] and cholesterol levels in blood [16]. Some pyridines constitute an important class of antitumor compounds [17-18]. They also show antibacterial [19], antifungal [20], antimyotic [21] and antidepressant [22] activities. Among them, 3-cyanopyridines have been identified as IKK- β inhibitors [23]. Most derivatives are prepared by manipulation of pyridine and its simple homologues in a manner similar to chemistry of the benzenoid chemistry. However the simple pyridine compounds are prepared by the cyclization of aliphatic raw materials. The pyridine nucleus is found in a large number of commonly used drugs which have diverse pharmacological activities. Interests in the synthesis of multicyclic pyridine containing compounds have increased in recent years because of their biological and pharmacological activities.

The structure of synthesized compounds were assigned based on Elemental analysis, IR ¹H-NMR and Mass spectral data. The antimicrobial activity was assayed by using the MIC (Minimum Inhibitory Concentration) method. All the compounds were screened in vitro for their antimicrobial activities against Gram +ve bacteria, Gram -ve bacteria and fungi. The biological activities of the synthesized compounds were compared with known standard drugs.

2. RESULTS AND DISCUSSION

The synthetic route adopted to obtain the 3-cyanopyridine derivatives 4a-4i and 5a-5i is shown in Scheme 1. 2-Amino-3-cyanopyridine derivatives 4a-4i were prepared from Chalcones 3a-3i by refluxing with malononitrile and ammonium acetate in Ethanol. The isolated product was crystallized with diethyl ether to get 2-amino-3-cyanopyridine derivatives in 58-83% yield. The 2-methoxy-3-cyanopyridine derivatives 5a-5i were prepared from Chalcones 3a-3i by refluxing with

malononitrile and sodium methoxide in ethanol for 8 hrs. The isolated products were crystallized with diethyl ether to get 2-methoy-3-cyanopyridine in 54-83% yield. The structures of all newly synthesized compounds were assigned on the basis of spectral data such as IR, $^1\text{H-NMR}$, Mass and elemental analysis.

Reagents and conditions: (a) 40% NaOH, Ethanol, R.T., 24 hrs
 (b) Malanonitrile, Ammonium formate, Ethanol, 80 °C, 8-12 hrs
 (c) Malanonitrile, Sodium methoxide, Ethanol, 80 °C, 8-12 hrs

Scheme 1: The Synthetic scheme for the preparation of compounds 4a-i and 5a-i.

The structural assignment of the title compounds 4a-i and 5a-i have been made on the basis of $^1\text{H-NMR}$, Mass, elemental analysis and IR spectral studies which were in full agreement with the proposed structures. IR spectrum of compound 4e reveals absorption band in the region 3322 cm^{-1} corresponding to amine (C-N) stretching and 2218 cm^{-1} due to -CN. In $^1\text{H-NMR}$ spectra of 4e, the -CHF₂ protons absorbed as a singlet at δ 7.01 and broad singlet at δ 5.98 due to amine for 2H proton and rest of the aromatic proton appear at their respective position. Mass spectrum of 2-amino-6-(4''-chlorophenyl)-4-[(4'-difluoromethoxy) (3'-hydroxy) phenyl] nicotinonitrile showed (M⁺) peak at 387.8 which support the formation of product. The structure of 5a is interpreted from spectroscopic data. The IR spectrum of 5e showed a characteristic absorption band at 2218 cm^{-1} due to -CN stretching and 1546 cm^{-1} due to vinyl (C=C) stretching. $^1\text{H-NMR}$ spectrum of 5g reveals the presence of the -CHF₂ protons absorbed as a singlet at δ 6.93 and methoxy group at δ 3.82. Mass spectrum of 6-(4''-methoxy phenyl)-4-[(4'-difluoro methoxy) (3'-hydroxy) phenyl] -2-methoxy nicotinonitrile showed (M⁺) peak at 447.2.

3. EXPERIMENTAL

All the melting points were determined on electro-thermal apparatus using open glass capillaries and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5mm thickness, and spots were located by iodine and UV (254nm). The IR spectra were recorded on a Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GCMS-QP2010 model using Direct Injection Probe technique. ^1H -NMR was determined in $\text{CDCl}_3/\text{DMSO}-d_6$ solution on a Bruker AC 400MHz spectrometer using TMS as internal standard and coupling constants (J) are expressed in Hertz (Hz). Elemental analysis of the all the synthesized compounds were carried out on Elementar Vario EL III Carlo Erba 1108 model, and the results are in agreements with the structures assigned. All the reagents were purchased from Rankem (New Delhi, India) and Sigma-Aldrich (New Delhi, India) and are used without further purification.

Procedure for synthesis of (E)-3-[4'-(difluoromethoxy)-3-hydroxyphenyl]-1-phenyl prop-2-en-1-one (3a)

To a solution of 4-(difluoromethoxy)-3-hydroxybenzaldehyde (0.01 mol) **1** in ethanol was added substituted Acetophenone (0.01 mol) **2** followed by catalytic amount of 40% aqueous NaOH solution and the reaction mixture was stirred for 5-6 hrs at room temperature. Completion of reaction checked by TLC. The reaction mixture was poured into crushed ice, filter and dried. The product was crystallized in ethanol. M.P. 160 °C. Yield; 90 %.

Similarly, other compounds (**3a-3i**) were synthesized and published.

Procedure of synthesis of 2-Amino-6-(4''-chlorophenyl)-4-[(4'-difluoro methoxy) (3'-hydroxy) phenyl] nicotinonitrile (4e)

To a solution of (E)-1-(4''-chlorophenyl)-3-[(4'-difluoromethoxy) (3'-hydroxy) phenyl]prop-2-en-1-one (0.5gm, 0.01mol) in ethanol (5 ml) was added malononitrile (0.12 gm, 0.01mol) and ammonium acetate (0.398gm, 0.03mol) is added in catalytically amount. Then reflux the reaction mixture at 80°C for 8 hrs. After completion of the reaction, the reaction mixture was poured into crushed ice water and product was extracted with ethyl acetate (20 ml). The organic layer was washed with brine, dry over sodium sulphate and evaporated under reduced pressure. The crude residue was washed with diethyl ether to a gives pure product as a solid. Yield 65%; m.p. 155 °C. IR (KBr, cm^{-1}): 3322 (-OH), 2951 (C-H str.)(asym), 2218 ($\text{C}\equiv\text{N}$ stretching) 1585 (C=C, stretching), 1116 (C-F) ; ^1H -NMR ($\text{DMSO}-d_6$, δ ppm): 5.98 (s, 2H, $-\text{NH}_2$), 7.01 (s, 1H, $-\text{CHF}_2$), 7.12-7.24 (t, 1H, $-\text{CH}$, aromatic), 7.26-7.34 (m, 1H, $-\text{CH}$, aromatic), 7.42-7.44 (d, 1H, $-\text{CH}$, aromatic), 7.49-7.51 (d, 2H, $-\text{CH}$, aromatic), 7.56-7.58 (d, 2H, aromatic), 7.60 (s, 1H, aromatic), 10.01(s, 1H, -OH). Mass: (m/z) 387.8. Anal. Calcd. for $\text{C}_{19}\text{H}_{12}\text{ClF}_2\text{N}_3\text{O}_2$: C: 58.85%, H: 3.12%, N: 10.84%; Found: C: 58.79%, H: 3.03%, N: 81.10 %.

Similarly, other 2-Amino-6-aryl-4-[(4'-difluoro methoxy) (3'-hydroxy) phenyl] nicotinonitrile compounds (**4a-4i**) were prepared. The physical data are recorded in Table no.: I.

Procedure of synthesis of 6-(4''-bromophenyl)-4-[(4'-difluoro methoxy) (3'-hydroxy) phenyl] -2-methoxy nicotinonitrile (5g)

To a solution of (E)-(4''-bromophenyl)-3-[(4'-difluoromethoxy)(3'-hydroxy) phenyl]prop-2-en-1-one (0.9gm, 0.01mol) in ethanol (5 ml) was added malononitrile (0.12 gm, 0.01mol) and sodium methoxide (0.07 gm, 0.01 mol) is added in catalytically amount. The reaction mixture was reflux at 80°C for 8 hrs. After completion of the reaction, the reaction mixture was poured into

crushed ice water and product was extracted with ethyl acetate (20 ml). The organic layer was washed with brine, dry over sodium sulphate and evaporated under reduced pressure. The crude residue was washed with diethyl ether to give pure product as a solid. Yield 68%; m. p. 148 °C. IR (KBr, cm^{-1}): 3364 (-OH), 2922 (C-H str.), 2218 ($\text{C}\equiv\text{N}$ stretching) 1546 (C=C, stretching), 1110 (C-F), 725 (C-Br); $^1\text{H-NMR}$ (DMSO-d_6 , δ ppm): 3.82 (s, 3H, - OCH_3), 6.93 (s, 1H, - CHF_2), 7.02-7.09 (t, 1H, -CH, aromatic), 7.17-7.19 (d, 1H, -CH, aromatic), 7.36-7.37 (d, 1H, -CH, aromatic), 7.40-7.49 (d, 3H, -CH, aromatic), 7.83-7.88 (d, 2H, aromatic), 10.09 (s, 1H, -OH). Mass: (m/z) 447.2. Anal. Calcd. for $\text{C}_{20}\text{H}_{13}\text{BrF}_2\text{N}_2\text{O}_3$: C: 53.71%, H: 2.93%, N: 6.26 %; Found: C: 53.69%, H: 2.84%, N: 6.13 %.

Similarly, other 6-Aryl-4-[(4'-difluoro methoxy) (3'-hydroxy) phenyl] -2-methoxy nicotinonitrile compounds (**5a-5i**) were prepared. The physical data are recorded in Table no.I.

Table no. I

Sr. No.	Ar	Molecular Formula	M.W.	M.P. °C	Yield %	% of Nitrogen	
						Calculated	Found
4a	C_6H_5 -	$\text{C}_{19}\text{H}_{13}\text{F}_2\text{N}_3\text{O}_2$	353	233	71	11.89	11.73
4b	4- CH_3 - C_6H_4 -	$\text{C}_{20}\text{H}_{15}\text{F}_2\text{N}_3\text{O}_2$	368	196	88	11.44	11.35
4c	4- OCH_3 - C_6H_4 -	$\text{C}_{20}\text{H}_{15}\text{F}_2\text{N}_3\text{O}_3$	383	117	83	10.96	10.89
4d	2- OCH_3 - C_6H_4 -	$\text{C}_{20}\text{H}_{15}\text{F}_2\text{N}_3\text{O}_3$	368	196	81	10.96	10.35
4e	4- Cl - C_6H_4 -	$\text{C}_{19}\text{H}_{12}\text{ClF}_2\text{N}_3\text{O}_2$	388	155	65	10.84	10.81
4f	4- F - C_6H_4 -	$\text{C}_{19}\text{H}_{12}\text{F}_3\text{N}_3\text{O}_2$	372	169	60	11.32	11.28
4g	4- Br - C_6H_4 -	$\text{C}_{19}\text{H}_{12}\text{BrF}_2\text{N}_3\text{O}_2$	432	149	70	9.72	9.61
4h	$\text{C}_4\text{H}_3\text{S}$ - 2-Thiophenyl	$\text{C}_{17}\text{H}_{11}\text{F}_2\text{N}_3\text{O}_2\text{S}$	360	180	58	11.69	11.54
4i	$\text{C}_5\text{H}_4\text{N}$ - 3-Pyridinyl	$\text{C}_{18}\text{H}_{12}\text{F}_2\text{N}_4\text{O}_2$	355	172	60	15.81	15.43
5a	C_6H_5 -	$\text{C}_{20}\text{H}_{14}\text{F}_2\text{N}_2\text{O}_3$	368	212	74	7.61	7.49
5b	4- CH_3 - C_6H_4 -	$\text{C}_{21}\text{H}_{16}\text{F}_2\text{N}_2\text{O}_3$	383	171	83	7.33	7.22
5c	4- OCH_3 - C_6H_4 -	$\text{C}_{21}\text{H}_{16}\text{F}_2\text{N}_2\text{O}_4$	399	163	80	7.03	6.87
5d	2- OCH_3 - C_6H_4 -	$\text{C}_{21}\text{H}_{16}\text{F}_2\text{N}_2\text{O}_4$	399	183	81	7.03	6.99
5e	4- Cl - C_6H_4 -	$\text{C}_{20}\text{H}_{13}\text{ClF}_2\text{N}_2\text{O}_3$	403	136	63	6.96	6.79

5f	4-F-C ₆ H ₄ -	C ₂₀ H ₁₃ F ₃ N ₂ O ₃	387	133	54	7.25	7.09
5g	4-Br-C ₆ H ₄ -	C ₂₀ H ₁₃ BrF ₂ N ₂ O ₃	448	148	68	6.26	6.13
5h	C ₄ H ₃ S- 2-Thiophenyl	C ₁₈ H ₁₂ F ₂ N ₂ O ₃ S	375	149	58	7.48	7.29
5i	C ₅ H ₄ N- 3-Pyridinyl	C ₁₉ H ₁₃ F ₂ N ₃ O ₃	370	178	62	11.38	11.23

4. ANTIMICROBIAL ACTIVITY

The newly synthesized compounds were screened for their antibacterial activity against Gram-positive (*S. aureus* ATCC 6538, *M. luteus* ATCC 9345), Gram negative (*E. coli* ATCC 4230, *S. thypi* ATCC 14028) bacteria, as described by the guidelines in NCCLS-approved standard document M7-A4, using the micro dilution broth procedure [24]. Ampicillin trihydrate was used as the reference antibacterial agent. The antifungal activities of the newly synthesized chemical compounds were tested against yeast strain (*C. albicans* ATCC 14053) according to the guidelines in NCCLS-approved standard document M27-A2, using the micro dilution broth procedure [25]. Fluconazole was used as the reference antifungal agent. The solutions of test compounds and reference drug were prepared by dissolving in DMSO at a concentration of 2560 µg/mL. The 2-fold dilutions of the compounds and the reference drug were prepared (1280, 640, 320, 160, 80, 40, 20, 10 µg/mL). Antibacterial activities of the newly synthesized chemical compounds were performed in Mueller-Hinton broth medium at a pH of 7.2 with an inoculum of $(1-2) \times 10^3$ cells/mL by the spectrophotometric method, and an aliquot of 100µL solution was added to each tube of serial dilution. The chemical compounds-broth medium serial tube dilutions inoculated with each bacterium were incubated on a rotary shaker at 37°C for 18 hr at 150 rpm. The minimum inhibitory concentration (MIC) of each chemical compound was recorded as the lowest concentration of each chemical compound in the tubes with no growth (i.e., no turbidity) of inoculated bacteria. Minimum inhibitory concentration (MIC, µg/mL) was measured and compared with control; the MIC values of the compound screened are given in below table no. II.

Table no. II

Comp. Id	Ar	Antibacterial Activity				Antifungal Activity
		Gram-positive bacteria		Gram-negative bacteria		
		<i>S. aureus</i>	<i>M. luteus</i>	<i>E. coli</i>	<i>S. thphi</i>	<i>C. albicans</i>
4a	C ₆ H ₅ -	80	80	40	40	40
4b	4- CH ₃ -C ₆ H ₄ -	160	160	160	80	80
4c	4-OCH ₃ -C ₆ H ₄ -	160	80	80	40	40
4d	2-OCH ₃ -C ₆ H ₄ -	80	80	160	40	40
4e	4-Cl-C ₆ H ₄ -	40	40	80	80	80
4f	4-F-C ₆ H ₄ -	160	80	80	160	160

4g	4-Br-C ₆ H ₄ -	80	80	40	40	40
4h	C ₄ H ₃ S- 2-Thiophenyl	40	40	80	80	80
4i	C ₅ H ₄ N- 3-Pyridinyl	160	80	80	80	80
5a	C ₆ H ₅ -	40	80	80	80	80
5b	4- CH ₃ -C ₆ H ₄ -	80	80	160	80	80
5c	4-OCH ₃ -C ₆ H ₄ -	160	160	80	80	80
5d	2-OCH ₃ -C ₆ H ₄ -	40	40	80	80	80
5e	4-Cl-C ₆ H ₄ -	40	40	40	80	80
5f	4-F-C ₆ H ₄ -	160	80	80	160	160
5g	4-Br-C ₆ H ₄ -	80	80	160	80	160
5h	C ₄ H ₃ S-2- Thiophenyl	40	40	40	80	80
5i	C ₅ H ₄ N- 3-Pyridinyl	80	80	80	40	40
Ampicillin	-	20	20	40	20	-
Fluconazole	-	-	-	-	-	10

From the result of biological evaluation, it has been observed that the compounds exhibited interesting biological activity, however with a degree of variation. Most of the compounds tested were found to have comparable antibacterial and exhibit low antifungal activity. From the table no. II, it can be observed that compounds 4a, 4c, 4d, 5a, 5b and 5i were moderate active against *S. aureus*, *M. luteus*, *Escherichia coli*, *S. thyphi.*, *C. albicans*, Compounds 4g, 4h, 5d, 5e and 5h were give promising activity against *S. aureus* A, *S. aureus*, *M. luteus*, *Escherichia coli*, *S. thyphi.*, *C. albicans*.

5. CONCLUSION

In summary, we have synthesized a series of novel 3-Cyanopyridines derivatives. All the newly synthesized compounds were confirmed with spectroscopic data like ¹H-NMR, Mass, IR Spectra, elemental analysis and evaluated antibacterial and antifungal activity. The antibacterial study shows that 3-Cyanopyridines derivatives showed moderate activity with MICs between 20 and 80 µg/mL. The 3-Cyanopyridines showed low antifungal activity. The importance of such work lies in the possibility that the new compounds might be more efficacious drugs against bacteria, which could be helpful in designing more potent antibacterial agent for therapeutic use.

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