

Synthesis and biological activity of novel benzothiazole pyridine derivatives

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Abstract: Potential biologically active derivatives of the 2-Amino-5-aryl or Formylpyridine-3-Carbonitriles (2a-d) were prepared by the cyclocondensation reaction of vinamidinium salts with malonitrile. The structures of these compounds were established on the basis of elemental (C, H, N) and spectral (¹H-NMR, ¹³C-NMR and spectral data) analysis. The compounds were screened for their antifungal and antibacterial activity. The compound 2-amino-5-formylpyridine-3-carbonitrile was found to be the most potent compound of series.

Keywords: Antibacterial and antifungal activity, Synthesis compounds, Vinamidinium salts.

I. Introduction:

Pyridine is a heterocyclic organic compound with the chemical formula C₅H₅N. It is structurally related to benzene, with one CH group replaced by a nitrogen atom. It is used as a precursor to agrochemicals and pharmaceuticals and is also an important solvent and reagent [1]. Pyridine was first isolated and characterized by Anderson in 1846. It was obtained from bone oil and from coal tar. The cyclic nature of pyridine was recognized by Korner and Dewar in 1869 [2].

Pyridine derivatives of different heterocyclic nucleus have shown potent pharmacological properties like antifungal [3-5], antibacterial [6], antimicrobial [7], and insecticidal [8]. In this connection, great attention has recently been paid for the synthesis of pyridine.

The pyridine ring is one of the most well-known systems among the naturally occurring hetero-cycles [9]. Classical methods for the formation of pyridines have been used extensively. Such methods include the Friedländer condensation of enamino ketones of β-ketoesters with 1,3-diketones [10], the Hantzsch pyridine synthesis from β-dicarbonyl compounds in the presence of an aldehyde and ammonia [11] and the related Knoevenagel cyclisation of β-aminoacrylates with α,β-unsaturated carbonyl compounds [12]. The synthesis of tri substituted pyridines has been reported [13-14] from the reaction of deoxybenzoin, vinamidinium species and ammonia in good yields and act as Cox-2 inhibitors. 2-Aminopyridine are promising substituted pyridines which have been shown to be biologically active molecules [15]. Additionally, because of their chelating abilities, 2-aminopyridines are commonly used as ligands in inorganic and organometallic chemistry [16]. This class of compounds and their derivatives are valuable synthetic target compounds and their synthesis has been extensively reviewed [17-18].

II. Experimental:

2.1 Reagents and analysis:

The solvents were purified according to standard procedures prior to use, and all commercial chemicals were used as received.

For thin-layer chromatography (TLC) was performed on pre-coated silica gel plates (0,25mm, Merck), Merck silica gel having size 0,063-0,200 mm was used for flash column chromatography. Melting point (mp) were determined in a capillary tube and are uncorrected ¹H NMR spectra were recorded on a Bruker AC 300MHz Spectrometer in CDCl₃ containing tetramethylsilane as an internal standard at room temperature at 400MHz.

Chemical shifts are reported in δ parts per million (ppm) downfield from tetramethylsilane (TMS) with reference to internal solvent and coupling constants in Hz. Elemental analyses were determined by using Perkin-Elmer 240c elemental analyzer.

2.2 General procedure:

The mixture of vinamidinium salt 1 (0.48 mmol), malononitrile (1 mmol), ammonium acetate (3.36 mmol), and anhydrous ethanol (8 mL) were combined in a reaction flask and allowed to reflux overnight at which time the reaction mixture was allowed to cool at room temperature and the solvent was removed by rotary evaporation. The resulting residue was partitioned between methylene chloride and water. The aqueous layer was extracted with fresh methylene chloride and the combined organic layers were dried over sodium sulphate. The drying agent was removed by filtration and the solvents were removed in vacuo to give the crude material. The crude product was purified by a column chromatography (10% EtOAc in hexane) to give product 2a–d. However, for trisubstituted pyridine 2d, after evaporation of the solvent, 6 mL of THF and 6 mL of 1 N HCl are added. The mixture is allowed to stir at room temperature for 2 h, then neutralized with saturated aqueous NaHCO₃ and extracted with three portions of methylene chloride.

2.3. Spectra data:

2 - Amino - 5 - phenylpyridine - 3 - carbonitrile (2a).

Yield: 96%; yellow solid; mp = 132–134°C; ¹H - NMR (300 MHz, CDCl₃): δ = 5.72 (s, 2H), 7.40–7.60 (m, 5H), 7.83–7.7.84 (d, 1H, J = 2.6 Hz), 8.41–8.42 (d, 1H, J = 2.6 Hz); Anal. calcd. for C₁₂H₉N₃: C, 73.83; H, 4.65; N, 21.52%. Found: C, 73.79; H, 4.60; N, 21.48%. Mass m/z (EI, 30 eV): m/z = 195 (M⁺).

2 - Amino - 5 - (4 - methoxyphenyl)pyridine - 3-carbonitrile (2b).

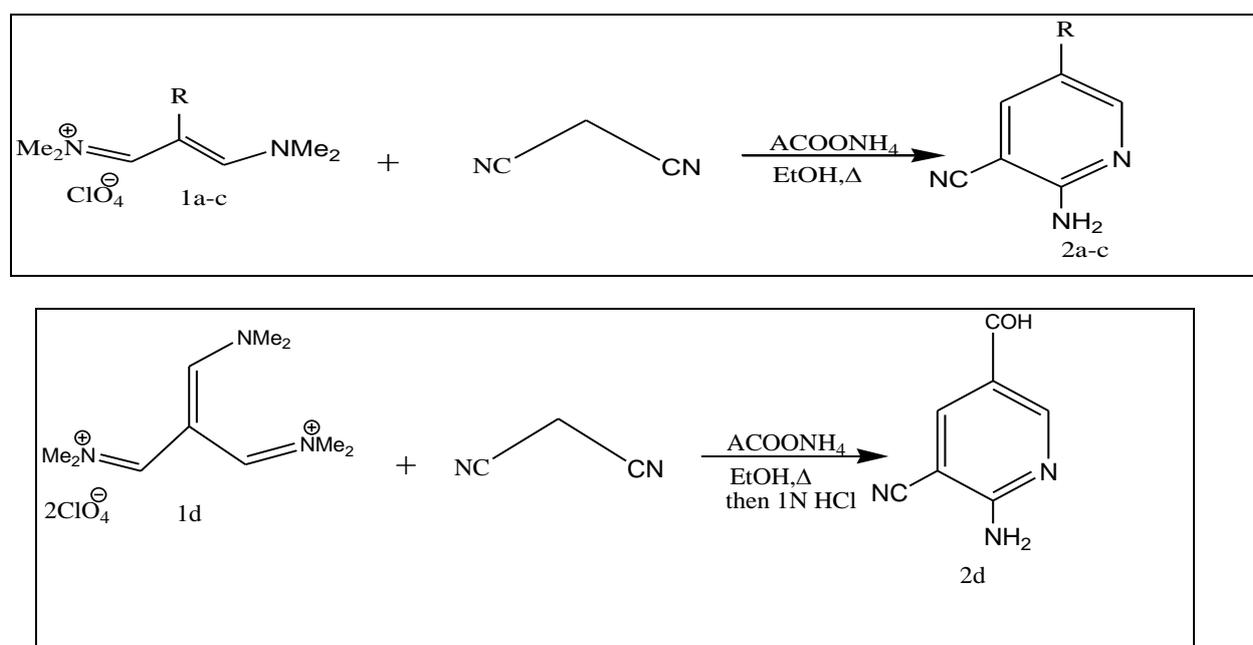
Yield: 97%; yellow solid; mp = 137–139°C; ¹H-NMR (300 MHz, CDCl₃): δ = 3.42 (s, 3H), 5.81 (s, 2H), 6.91–6.96 (m, 2H), 7.11–7.16 (m, 2H), 7.49–7.50 (m, 2H, J = 2.4 Hz), 7.94–7.95 (d, 1H, J = 2.4 Hz); ¹³C-NMR (CDCl₃) δ = 55.8, 107.6, 113.9, 128.8, 132.6, 132.8, 135.8, 136.2, 137.1, 145.7, and 159.9. Anal. calcd. for C₁₃H₁₁N₃O: C, 69.32; H, 4.92; N, 18.65%. Found: C, 69.28; H, 4.89; N, 18.62%. Mass m/z (EI, 30 eV): m/z = 225 (M⁺).

2-Amino-5-(1-naphtyl)pyridine-3-carbonitrile (2c).

Yield: 92%; yellow solid; mp = 148–150°C; ¹H-NMR (300 MHz, CDCl₃): δ = 6.51 (s, 2H), 7.40–7.87 (m, 5H), 7.83–7.84 (d, 1H, J = 2.4 Hz), 8.41–8.42 (d, 1H, J = 2.4 Hz); ¹³C-NMR (CDCl₃) δ = 125.53, 126.157, 127.45, 128.45, 129.189, 130.30, 130.95, 132.8, 135.8, 136.2, 137.1, 145.7, and 159.9. Anal. calcd. for C₁₆H₁₁N₃: C, 78.35; H, 4.52; N, 17.13%. Found: C, 78.31; H, 4.49; N, 17.11%. Mass m/z (EI, 30 eV): m/z = 245 (M⁺).

2-Amino-5-formylpyridine-3-carbonitrile (2d).

Yield: 78%; yellow-white solid; mp = 127–129°C; ¹H-NMR (300 MHz, CDCl₃): δ = 5.06 (s, 2H), 8.18 (d, H, J = 2.3 Hz), 9.21(d, H, J = 2.3 Hz), 9.95 (s, 1H). ¹³C-NMR (CDCl₃) δ = 97.31, 115.38, 123.24, 142.19, 154.92, 168.84, and 192.45. Anal. calcd. for C₇H₅N₃O: C, 57.14; H, 3.43; N, 28.56%. Found: C, 57.11; H, 3.41; N, 28.53%. Mass m/z (EI, 30 eV): m/z = 147 (M⁺).



Scheme 1

Table 1:

Synthesis of 2-amino -5-aryl or formylpyridine -3-carbonitriles 2a-d

Compounds	R	R'	Recrystallisation solvent	Yield %
2a	Ph	Ph	éthanol	96
2b	4- CH ₃ Ph	4- CH ₃ Ph	éthanol	97
2c	Naphtyl	Naphtyl	éthanol	92
2d	\ominus HC=NMe ₂	CHO	hexane	78

All products were characterized from their ¹HNMR, ¹³CNMR and mass spectroscopic data

2.4. Antimicrobial activity assays:

All the compounds (2a-d) were screened for their antibacterial activity against four bacteria: *Vibrio alginoliticus*, *Vibrio cholera*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* and for their antifungal activity against four *Candida* species as *Candida parapsilosis*, *Candida krusei*, *Candida glabrata* and *Candida albicans*.

2.5. Antibacterial activity tests: Screening for anti-Vibrio spp. activity

The quantitative anti-Vibrio spp. assay of compounds (2a-d) was carried out by the disc diffusion method described by Gulluce[19] and modified in this work. The overnight bacterial cultures on a glass tube containing 9 ml of alkaline peptone water (1% NaCl, pH: 8.4) at 37 °C were used for the antimicrobial activity of the compounds (2c, 2d) and the optical density was adjusted at 0.5 McFarland turbidity standards with a DENSIMAT (Biomerieux). The inoculums of the respective bacteria were streaked onto MHI agar supplemented with 1% NaCl using a sterile swab. The test compounds were dissolved in dimethyl sulphoxide (DMSO) to obtain 1 mg/ml stock solutions. The discs (diameter 6 mm, Whatman paper No. 3) were respectively impregnated with 10 µl/disc of each of the tested compounds stock solutions and placed onto the agar plates. The dishes were incubated at 37°C for 18–24 h. The diameter of the zones of inhibition around each of the discs was taken as measure of the antimicrobial activity. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded. Tetracycline (30µg/disc) was used in this study as positive control. The antibiotic susceptibility was determined by using the Kirby-Bauer method and Muller Hinton agar plates supplemented with 1% NaCl as described by Ottaviani [20]. After incubation at 37°C for 18 to 24 h, the diameter of the inhibition zone was measured with 1 mm flat rule [21].

2.6. Evaluation of antifungal activity:

The same agar-disc diffusion method was used for screening the antifungal activity of tested compounds. The four *Candida* species were grown on Sabouraud chloramphenicol agar plate at 30°C for 18–24 h. Several colonies of similar morphology of the clinical yeast were transferred into Api suspension medium and adjusted to 2 McFarland turbidity standard with a Densimat (BioMerieux). The inocula of the respective yeast was streaked on to Sabouraud chloramphenicol agar plates at 30 °C using a sterile swab and then dried. A sterilized 6 mm paper disc was loaded with 10 ml (1 mg/ ml) of each of the tested compounds dissolved in dimethyl sulphoxide. The treated Petri dishes were placed at 4°C for 1–2 h and then incubated at 37°C for 18–24 h. The inhibition of fungal growth was also evaluated by measuring the diameter of the transparent inhibition zone around each disc. The average of three measurements was taken. The susceptibility of the standard was determined using a disc paper containing 20 mg of amphoterecin B.

III. Results And Discussion:

We report here a simple method for the synthesis of 2-amino-5-aryl or formylpyridine-3-carbonitriles in one-step procedure by condensation of symmetrical vinamidinium salts with malononitrile and ammonium acetate.

All of these salts, were isolated as the perchlorate and were used without further purification for the synthesis of 3,5-disubstituted 2-aminopyridines. As shown in Scheme 1, the symmetrical vinamidinium salts (1a–d) were allowed to react with malononitrile in refluxing ethanol in the presence of ammonium acetate for 12 h to afford the 2-amino-5-aryl or formylpyridine-3-carbonitriles (2a–d). For the most part the reactions were rather clean and proceed in excellent yields (Table 1).

The study of antimicrobial activities of the synthesized compounds showed significant biological activities (table 2).

All compounds showed antibacterial activity. On the other hand, the best activities were found with the compound 2d, in fact, the activity of this compound is similar to that of Tetracycline, which explains the importance of this compound.

Table 2: Zones of growth inhibition (mm) showing antibacterial activity of four tested compounds against *Vibrio* spp.

Strains	Zones of growth inhibition (mm)				
	1	2	3	4	Tetracycline
<i>Vibrio alginoliticus</i>	15.2±1.8	16.1± 1.2	16.3±1.7	19.3± 1.3	27.5 ± 3.8
<i>Vibrio cholera</i>	14.8±1.6	15.4± 1.5	15.2±1.2	21.4± 1.	24.8±4.4
<i>Vibrio parahaemolyticus</i>	13.7±1	14.2± 1.7	14.2±1.1	18.2± 1.7	26.1 ± 2.9
<i>Vibrio vulnificus</i>	14.6±1.9	15.5± 2.2	15±1.5	22.8± 2.1	25.9 ± 3.5

Similarly for the antifungal activity, the results are presented in Table 3. All tested compounds showed significant antifungal activity. These activities were variable depending on the fungal species and the tested compound. The best activities were obtained with the compound 2d, but in general the activity of all compounds was lower than Amphoterecin B.

As shown in table 2 and 3, compound 2d was the most potent inhibitory for the growth of fungi and bacteria. On the other hand, this compound has an aldehyde function, which explains its high biological activities.

Our obtained results were in agreement with the literature. In fact, antimicrobial activities of pyridine compounds were reported by Muthal [22].

Table 3: Zones of growth inhibition (mm) showing antifungal activity of four tested compounds against four *Candida* spp

Fungi	Zones of growth inhibition (mm)				
	1	2	3	4	Amphoterecin B.
<i>Candida parapsilosis</i>	5±0.2	6.3± 0.3	5.5±0.2	9± 1.1	16±1.1
<i>Candida krusei</i>	7.5±0.8	9.1± 0.7	12±0.2	15± 0.9	21±1.1
<i>Candida glabrata</i>	7.5±0.4	7.2± 0.5	5±0.3	9.1± 0.5	13±0.9
<i>Candida albicans</i>	6.5±0.2	7.4± 0.3	6.5±0.6	10.7± 0.4	25±1

IV. Conclusion

In summary, we have demonstrated that malononitrile react with vinamidinium salts in the presence of ammo-nium acetate to give in one-step the corresponding 2- amino-5-aryl or formylpyridine-3-carbonitriles in fair to excellent yields. All the synthesized compounds were evaluated for their antimicrobial activity. The investigation of antimicrobial and antifungal screening data revealed that all the tested compounds showed moderate to significant activity.

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