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Antibacterial activity of 2-amino-3-cyanopyridine derivatives

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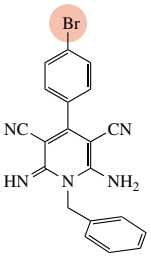
New 2-amino-4-aryl-3-cyanopyridines were obtained by the three-component condensation of arylidenemalononitriles, malononitrile and (di)amine system or acetoacetanilide. Antibacterial activity of all synthesized compounds against the Gram-negative bacteria *E. coli* and the Gram-positive *B. subtilis* was tested, the only representative showed a substantial antimicrobial effect.

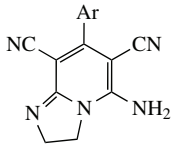


Keywords: 3,5-dicyanopyridines, antimicrobial activity, halogen bonding, bacteria, *E. coli*, *B. subtilis*.

Pyridine ring, an important structural fragment in heterocyclic chemistry, is present in many natural products such as nicotinic acid, nicotinamide, vitamin B_6, which play key roles in metabolism. Functionalized pyridines exhibit a broad range of biological activities.[1–10] Antimicrobial resistance is currently one of the biggest threats to human health and development. Besides the optimization of the use of the current antimicrobial medicines, prevention to reduce the incidences of infections and improve public awareness, the development of new pharmaceuticals preferably acting in new ways is crucial. The seriousness of this is well-reflected by the resistance to penicillin reaching up to 51% of the infections, and 8–65% of *E. coli* associated with urinary tract infections being resistant to ciprofloxacin.[11] Accordingly, the term 'extreme drug resistance' has been coined to describe isolates of clinically-important species such as *Enterobacteriaceae* that are resistant to virtually all currently used antibiotics.[12] Antibiotic resistance is currently associated with 700 000 deaths yearly, and is projected to cause extra 10 million deaths a year worldwide by 2050, of which over 4 million cases yearly are expected in Africa and overall 90% in developing countries.[13]

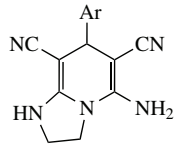
According to the concise literature survey above, the three-component reaction of arylidenemalononitriles, malononitrile and amines lead the formation of 2-amino-4-aryl-3-cyano-pyridines **1–6** in good yields, under mild conditions. The synthesis of compounds **1a–g**, **2a**,**b** and **3a–d** has been reported earlier,[14] with the structures of **1b**,**d**,**e** and **2b** having been confirmed by single crystal X-ray diffraction. Compounds **4–6** obtained in this work have not been described previously. Their synthesis is similar to that of their analogues[14] and is given in Online Supplementary Materials.

When arylidenemalononitriles with electron-donating (Me, OMe and NMe_2) and electron-acceptor (F and Br) substituents were applied, final oxidation lead to dihydroimidazopyridines **1a–g**. Surprisingly, when arylidenmalononitriles bearing aryl with greater electron-withdrawing effect (2,4- or 2,6-dichloro-phenyl) were used, tetrahydroimidazopyridines **2a**,**b** were obtained as the major products. Formation of 2-imino-1,2-di-hydropyridines **3a–d** took place when benzylamine was used instead of ethylenediamine (see ref. 14 and Scheme S1 in Online Supplementary Materials). The use of (2-thienyl)methylamine in place of benzylamine resulted in formation of non-oxidized 2-amino-1,4-dihydropyridine system **4**.[†]



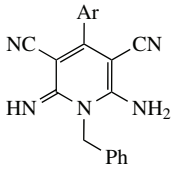
1a–g

a Ar = Ph
b Ar = 4-MeC_6H_4
c Ar = 4-BrC_6H_4
d Ar = 4-FC_6H_4
e Ar = 4-$MeOC_6H_4$
f Ar = 4-$Me_2NC_6H_4$
g Ar = 3-MeO-4-HOC_6H_3



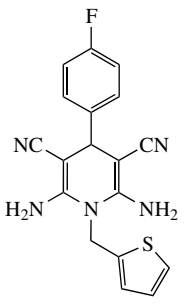
2a,**b**

a Ar = 2,4-$Cl_2C_6H_3$
b Ar = 2,6-$Cl_2C_6H_3$

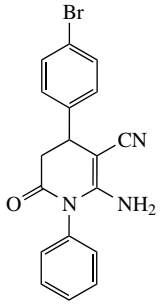


3a–d

a Ar = Ph
b Ar = 4-$MeOC_6H_4$
c Ar = 4-BrC_6H_4
d Ar = 4-$F_3CC_6H_4$



4



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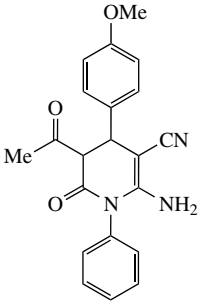

6

According to the concise literature survey above, the three-component reaction of arylidenemalononitriles, malononitrile and amines lead the formation of 2-amino-4-aryl-3-cyano-pyridines **1–6** in good yields, under mild conditions. The synthesis of compounds **1a–g**, **2a**,**b** and **3a–d** has been reported earlier,[14] with the structures of **1b**,**d**,**e** and **2b** having been confirmed by single crystal X-ray diffraction. Compounds **4–6** obtained in this work have not been described previously. Their synthesis is similar to that of their analogues[14] and is given in Online Supplementary Materials.

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Herein we found that condensation of acetoacetanilide with 4-bromobenzylidenemalononitrile (Scheme S2, Online

Supplementary Materials) in the presence of the catalytic amount of piperidine/piperazine was accompanied by deacetylation leading to tetrahydropyridine **5**. The use of 4-methoxybenzylidenemalononitrile with electron-donating substituent in the same reaction gives product **6** with retained acetyl group.

Previously, 2-amino-3-cyanopyridines have been studied for a variety of bioactivities, involving anticancer and acetylcholine esterase inhibitory activity, and as anti-inflammatory agents.[15–17] Amongst the synthetic approaches to 2-amino-3-cyanopyridines those involving multicomponent one-pot reactions forming the pyridine ring from acyclic precursors are most challenging.[15] High yielding synthetic approaches involving malonitrile, aromatic aldehydes and amines have recently been developed, facilitating access to a diverse substituent pattern.[18,19]

The herein considered cyanopyridines **1**–**6** were tested (at single concentration of 200 μg ml^{-1}) against *Escherichia coli* (Gram-negative) and *Bacillus subtilis* (Gram-positive) bacteria. Of these, only compound **3c** showed substantial antimicrobial effect, and it was retested at different concentrations to determine IC values. Against *E. coli*, the found MIC value was 577 μg ml^{-1}, and against *B. subtilis* it was 288 μg ml^{-1}. For cytotoxicity, we found EC_{50} of 95 μg ml^{-1} against the human MCF-7 cell line.

The observation that compound **3c** encompassing a bromine, but not compound **3a** with a hydrogen at the same position, exhibits antibacterial activity suggested that the bromine participates in a hydrophobic interaction, acts either as hydrogen bond acceptor or as a halogen bond donor. The fact that neither **3b** nor **3d** showed activity against *E. coli* or *B. subtilis* indicates that hydrogen bonding at this position is unlikely to be of impact for the antibacterial activity of **3c**, whereas the comparable polarity of the OMe-substituted **3b** and the Br-substituted **3c** suggested hydrophobicity do not play an important role. Thus, halogen bonding, which has lately been demonstrated to be often beneficial for bioactivity, is most likely involved in the antibacterial activity of **3c**.[20,21]

It should be noted that compounds **1c** and **5**, also bearing 4-bromophenyl residue yet missing an *N*-benzyl substituent lacked antimicrobial activity. It is therefore tempting to assume that the antimicrobial effect of **3c** also depends on a lipophilic interaction *via* the *N*-benzyl group, which due to steric reasons might not be available for a phenyl substituent of **5**. The lack of activity of **1c** may also depend on the unfavorable steric or electronic effect of its 4,5-dihydro-1*H*-imidazole functionality.

In summary, various 2-amino-3-cyanopyridines are readily accessible *via* a one-pot multicomponent reaction. The evaluation of the antimicrobial activity of a series of derivatives resulted in the identification of a compound with promising activity against both Gram-positive and Gram-negative bacteria. The lack of activity of structurally closely related compounds suggests halogen bonding to likely play an important role in the bioactivity, and the importance of the presence of the *N*-benzyl substituent. Further studies of 2-amino-3-cyanopyridines are expected to provide new antibacterial leads along with the understanding of the mechanism of their action.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.07.031.

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† *2,6-Diamino-4-(4-fluorophenyl)-1-(thiophen-2-ylmethyl)-1,4-dihydropyridine-3,5-dicarbonitrile* **4**. A mixture of 2-(4-fluorobenzylidene)-malononitrile (5.1 mmol) and malononitrile (5.2 mmol) were dissolved in MeOH (35 ml), and this was stirred for 5–7 min. (2-Thienyl)methylamine (5.2 mmol) was added under vigorous stirring (TLC control, EtOAc/hexane, 2 : 1). The reaction was allowed to proceed for 48–72 h. Upon evaporation of the solvent, the product crystallized and the crystals precipitated. The crystals were filtered off using filter paper and recrystallized from a 3 : 2 EtOH/H_2O mixture to afford product **4** in 84% yield, mp 205 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.96 (s, 1H, CH-10), 5.17 (s, 2H, CH_2-6), 6.37 (s, 4H, $2NH_2$-13/22), 6.65–7.52 (m, 7H, H17–21, H18–20, H-3, H-4, H-2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 39.3 (CH-10), 42.9 (CH_2-6), 63.1 (C-9 and C-11), 115.1 (d, $^2J_{C,F}$ 22.5 Hz, C-18 and C-20), 121.6 (CN-14 and CN-15), 126.9 (C-3), 127.4 (C-4), 128.2 (C-2), 128.85 (d, $^3J_{C,F}$ 7.5 Hz, C-17 and C-21), 137.6 (C-5), 141.7 (d, $^4J_{C,F}$ 3.0 Hz, C-16), 152.5 (C-8 and C-12), 161.3 (d, $^1J_{C,F}$ 240.8 Hz, C-19). HRMS, *m/z*: 352.1036 (calc. for $C_{18}H_{14}FN_5S$, *m/z*: 352.0954 [M+H]$^+$).