

Synthesis of novel 3-pyridinecarbonitriles with amino acid function and their fluorescence properties

A. S. Girgis¹, A. Kalmouch², and H. M. Hosni¹

¹ Pesticide Chemistry Department, National Research Centre, Dokki, Cairo, Egypt

² Tanning Materials and Protein Chemistry Department, National Research Centre, Dokki, Cairo, Egypt

Received August 5, 2003

Accepted October 22, 2003

Published online December 31, 2003; © Springer-Verlag 2003

Summary. A variety of *N*–[(4,6-diaryl-3-pyridinecarbonitrile)-2-yl] amino acid esters **2–4** were synthesized through the reaction of 2-bromo-3-pyridinecarbonitriles **1** with the appropriate α -amino acid ester hydrochloride in refluxing dioxane in the presence of triethylamine as dehydrohalogenating agent. Similarly, *N*–glycylglycine analogues **5** were obtained through the reaction of **1** with the dipeptide ester. On the other hand, attempts were made towards the construction of amino acid derivatives **7** through the reaction of **1** with aqueous solution α -amino acids **6** in refluxing pyridine, but were unsuccessful, and instead the unexpected 2-amino-3-pyridinecarbonitriles **8** were isolated. The fluorescence properties of the newly synthesized pyridines **2–5** were evaluated. Some of the prepared compounds show considerable antibacterial activity.

Keywords: 3-Pyridinecarbonitriles – α -Amino acids – Aromatic nucleophilic substitution – Fluorescence properties

Introduction

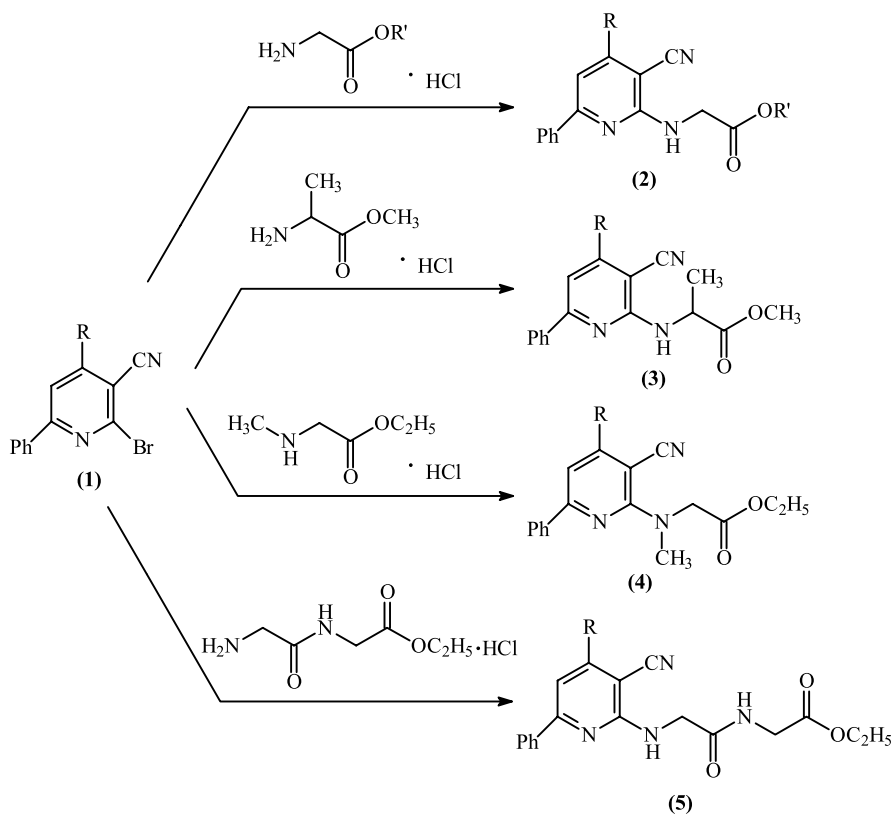
3-Pyridinecarbonitrile derivatives represent an important class of heterocyclic compounds characterized by highly pronouncing biological properties such as antiviral (Ibrahim et al., 1995; Koeckritz et al., 1992; Kul'nevich et al., 1990), antibacterial (Ghoneim et al., 1988; Goda, 1999; Khalil et al., 1990; Moustafa et al., 1992; Omar et al., 1996; Prakash et al., 1988; Shah et al., 1996), antifungal (Khalil et al., 1990; Omar et al., 1996; Schubert et al., 1990; Shah et al., 1996), herbicidal (Drumm et al., 1992, 1995; Hegde et al., 1991; Kunz et al., 1999) and orthopodocidal (Frasier et al., 1997), in addition to antitumour (Gaeta et al., 1998a, b; Hughes et al., 1990), anti-inflammatory (Monna et al., 1999; Weier et al., 1996) as well as analgesic and antihypertensive (Ebeid et al., 1991; Faermark et al., 1990; Mezentseva et al., 1991) activity. From all the above reports and in continuation with our previous work concerning the construction of various 3-pyridine-

carbonitrile containing compounds (Mishriky et al., 1994, 1997, 2000), it is intended in the present work to investigate the synthesis of novel 3-pyridinecarbonitrile derivatives with α -amino acid and/or peptide function as a natural product residue. The fluorescence properties of the newly synthesized compounds will be evaluated. Also, the antimicrobial activity against a variety of Gram +ve, Gram –ve bacteria and yeast will be screened.

Results and discussion

Reaction of 2-bromo-4,6-diaryl-3-pyridinecarbonitriles **1a–c** with either glycine methyl or ethyl ester hydrochlorides in refluxing dioxane in the presence of triethylamine as dehydrohalogenating agent, underwent aromatic nucleophilic substitution affording the corresponding *N*–[(4,6-diaryl-3-pyridinecarbonitrile)-2-yl]glycine esters **2a–e**. The structure of the latter was inferred from different spectroscopic (IR, ¹H-NMR, MS) as well as elemental analyses data. The IR spectra of **2a–e** exhibit in addition to the nitrile stretching vibration band ($\nu = 2215\text{--}2204\text{ cm}^{-1}$), the carbonyl ester vibration band ($\nu = 1751\text{--}1739\text{ cm}^{-1}$). ¹H-NMR spectra reveal the ester function (singlet at $\delta = 3.80\text{--}3.81$ in case of methyl esters **2a,c** and triplet at $\delta = 1.30\text{--}1.32$ as well as quartet at $\delta = 4.26\text{--}4.27$ in case of ethyl esters **2b,d,e**), in addition to the triplet NH residue ($\delta = 5.88\text{--}5.95$) due to its mutual coupling with the adjacent glycinate methylene protons ($\delta = 4.36\text{--}4.41$). The pyridine H-5 appears as a singlet signal at $\delta = 7.18\text{--}7.23$.

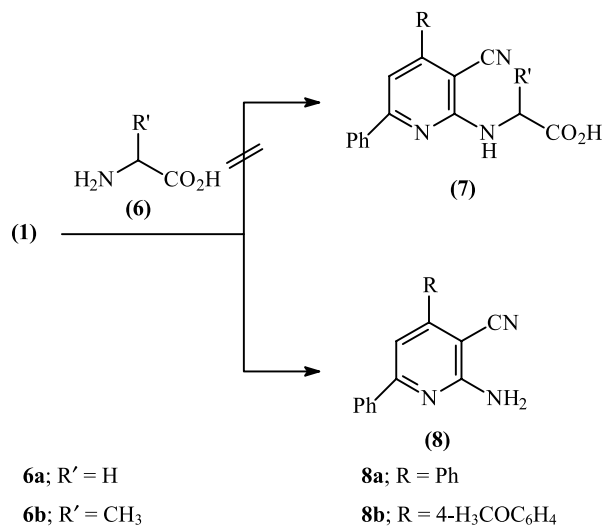
Similarly, reaction of **1b,c** with alanine methyl ester hydrochloride under the same reaction conditions gave the corresponding *N*–[(4,6-diaryl-3-pyridinecarbonitrile)-

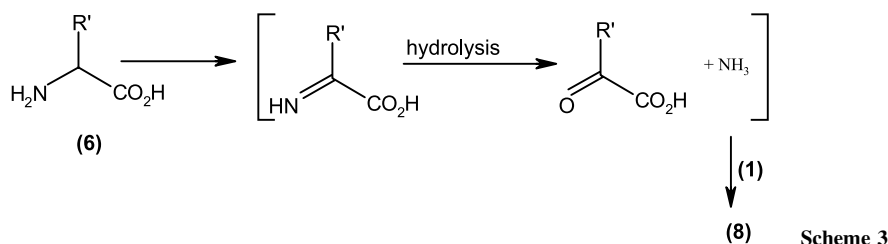
**1a;** R = Ph**1b;** R = 4-ClC₆H₄**1c;** R = 4-H₃COC₆H₄**3a, 4a, 5a;** R = 4-ClC₆H₄**3b, 4b, 5b;** R = 4-H₃COC₆H₄**2a;** R = Ph, R' = CH₃**2b;** R = Ph, R' = C₂H₅**2c;** R = 4-ClC₆H₄, R' = CH₃**2d;** R = 4-ClC₆H₄, R' = C₂H₅**2e;** R = 4-H₃COC₆H₄, R' = C₂H₅**Scheme 1**

2-yl]alanine methyl esters **3a,b**. Also, *N*-[(4,6-diaryl-3-pyridinecarbonitrile)-2-yl]sarcosine ethyl esters **4a,b** were obtained through the reaction of **1b,c** with sarcosine ethyl ester hydrochloride “as a representative example of secondary α -amino acid ester”.

Meanwhile, reaction of **1b,c** with *N*-glycylglycine ethyl ester hydrochloride “as a representative example of dipeptide derivative” yielded the corresponding *N*-[(4,6-diaryl-3-pyridinecarbonitrile)-2-yl]-*N'*-glycylglycine ethyl esters **5a,b**. ¹H-NMR spectra of **5a,b** exhibit in addition to the ethyl ester group (triplet at $\delta = 1.17$ – 1.22 , quartet at $\delta = 4.10$ – 4.15 assignable for the methyl and methylene ester protons, respectively), the two glycinate methylene protons ($\delta = 4.01$ – 4.07 and $\delta = 4.30$ – 4.35) (Scheme 1).

On the other hand, attempts were made towards the construction of *N*-[(4,6-diaryl-3-pyridinecarbonitrile)-2-

**Scheme 2**



yl]amino acids **7** through the reaction of **1** with aqueous solution α -amino acids **6** in refluxing pyridine, but were unsuccessful and instead the unexpected 2-amino-3-pyridinecarbonitrile derivatives **8** were isolated (Scheme 2). Formation of **8** through the reaction of **1** with **6** could be explained analogously to the famous ninhydrin reaction with α -amino acids (Greenstein et al., 1961). Where, the amino acid gave the corresponding imino acid under the applied reaction conditions. The latter upon hydrolysis liberated ammonia, which in turn interacted with **1** affording eventually **8** (Scheme 3). Actually, up to our knowledge the formation of 2-amino-3-pyridinecarbonitriles through the reaction of 2-halo-3-pyridinecarbonitrile derivatives with amino acids is unprecedented. The structure of **8a,b** was established through IR, ^1H -NMR, MS as well as elemental analyses data. ^1H -NMR spectra of **8a,b**

reveal the amino protons as a singlet signal ($\delta = 5.36$, 5.32 for **8a** and **8b**, respectively), in addition to the pyridine H-5 singlet signal ($\delta = 7.21$, 7.19 for **8a** and **8b**, respectively). The IR spectra of **8a,b** show a strong nitrile stretching vibration band ($\nu = 2206\text{ cm}^{-1}$).

Fluorescence properties of the prepared 3-pyridinecarbonitriles

The fluorescence properties of the newly synthesized 3-pyridinecarbonitriles were determined and the obtained results of Φ_s "quantum yield of the tested compounds" were measured relative to quinine sulfate used as a reference standard applying low concentrations to avoid reabsorption. The following relation was applied to calculate the fluorescence quantum yields (Φ_s) relative to the

Table 1. Absorption, excitation and emission properties of the prepared compounds in chloroform

Compd. no.	Absorption		Excitation λ_{max} (nm)	Emission λ_{max} (nm)	Φ_s^*
	λ_{max} (nm)	ξ_{max}			
2a	260	28404	265	405	0.526
	355	13039	364		
2b	260	28866	264	408	0.487
	356	13746	366		
2c	264	28408	268	410	0.310
	358	12109	367		
2d	264	29962	269	410	0.471
	358	13061	367		
2e	265	29140	270	404	0.428
	356	14030	366		
3a	266	32299	270	410	0.435
	358	13415	367		
3b	266	29898	270	406	0.603
	356	14095	367		
4a	271	39199	274	425	0.156
	366	11865	371		
4b	273	37914	275	416	0.088
	365	13744	371		
5a	265	35525	269	413.5	0.240
	356	14028	365		
5b	265	31267	270	407	0.399
	355	15433	362		

* Φ_s : quantum yield of the tested compound measured relative to quinine sulfate used as a reference standard using the lowest wave length excitation maximum of the tested compound

fluorescence quantum yield of quinine sulfate ($\Phi_r = 0.546$ in 0.1N H_2SO_4 solution) using the lowest wave length excitation maxima of the tested compounds (Basta et al., 2002; Morris et al., 1976; Parker, 1968).

$$\Phi_s = \Phi_r \frac{F_s q_r A_r n_s^2}{F_r q_s A_s n_r^2}$$

Where; F_s , F_r are the relative fluorescence of the determined compound and reference standard, respectively; q_s , q_r are the relative photon output of the source at the excitation wave length for the tested compound and reference standard, respectively; A_s , A_r are the absorbance at the excitation wave length for the tested compound and the reference standard, respectively; n_s , n_r are the refractive index for solvents used to dissolve the tested compound and reference standard, respectively.

From the data obtained (Table 1), it is obvious that, all the prepared 3-pyridinecarbonitriles **2–5** show two main excitation bands at $\lambda = 264–275$ and $362–371$ nm with respect to the two UV absorption maxima appeared at $\lambda = 260–273$, $355–366$ nm regions. Only one emission band is observed at $\lambda_{max} = 404–425$ nm at any of the observed excitation bands.

All the tested pyridine derivatives show considerable fluorescence properties with remarkable quantum yield. Meanwhile, compounds **4a,b** reveal the lowest quantum yield fluorescence activity compared with the other prepared pyridines **2**, **3**, **5**. This could be attributed to the effect of sarcosine moiety attached to the pyridine nucleus at the 2-position. In other words, it could be concluded that the attachment of secondary α -amino acid function to the active fluorescence system decreases the total quantum yield of the obtained compound compared with the primary α -amino acid or peptide residue.

Antimicrobial activity of the synthesized compounds

The antimicrobial activity of the synthesized compounds was tested by the disk diffusion method (Mishriky et al., 2001) using Whatman No. 1 filter paper disks (0.5 cm diameter). A solid Mueller Hinton Agar medium was sterilized and divided while hot ($40–50^\circ C$) in 15 ml portions in sterile petri-dishes. One ml of cell suspension (2×10^8 cells/ml) of exponential phase cultures of Gram-positive bacterial (*Staphylococcus aureus*, *Coagulase negative Staphylococcus* and β -Hemolytic streptococcus “group A”), Gram-negative bacterial (*Proteus spp.*, *Klebsiella spp.* and *Escherichia coli*) and yeast (*Candida albicans*) strains being tested was spread all over the surface of the solid medium.

The sterile disks were impregnated with different compounds ($200 \mu g/\text{disk}$). The saturated disks were placed carefully on the surface of the inoculated solid medium and the petri-dishes were incubated at $5^\circ C$ for 1 h to permit good diffusion and then transferred to an incubator at $37^\circ C$, $28^\circ C$ for the tested bacterial and yeast strains, respectively.

The results were recorded by measuring the inhibition zones caused by the various compounds on the tested microorganisms after 24 h of incubation. Tetracycline hydrochloride was used as a reference standard in this investigation. From the results obtained it has been found that, most of the tested compounds (**1a,c**; **2c,e**; **3a,b**; **4a,b** and **8a**) show no activity against the tested microorganisms at the used concentration ($200 \mu g/\text{disk}$). However, few compounds (**2a**, **2b**, **2d**, **5a** and **8b**) “Table 2” exhibit considerable activity (weak to moderate activity) against *Staphylococcus aureus* (Gram-positive bacterium). Only

Table 2. Antimicrobial activity of the prepared compounds

Microorganisms* Compd. no.	1	2	3	4	5	6	7
1b	—	+	—	—	—	—	—
2a	+	—	—	—	—	—	—
2b	+	—	—	—	—	—	—
2d	++	—	—	—	—	—	—
5a	+	—	—	—	—	—	—
5b	—	—	—	+	—	—	—
8b	++	—	—	—	—	—	—
Ref. **	+++	++	++	++	++	++	—

* 1: *Staphylococcus aureus*; 2: *Coagulase negative Staphylococcus*; 3: β -Hemolytic streptococcus (group A);

4: *Proteus spp.*; 5: *Klebsiella spp.*; 6: *Escherichia coli*; 7: *Candida albicans*

** Ref.: Tetracycline hydrochloride

+++ : Highly active (>12 mm); ++ : Moderately active (9–12 mm); + : Slightly active (5–9 mm);

— : Inactive

5b reveals weak antibacterial activity against *Proteus spp.* (Gram-negative bacterium). These results indicate that, the type of amino acid side chain attached to the pyridinecarbonitrile nucleus is the controlling factor responsible for the antimicrobial properties of the tested compounds. Generally, the biologically active compounds can be described more or less, as narrow spectrum Gram positive antibacterial agents.

Experimental

Melting points are uncorrected and recorded on an Electrothermal 9100 melting point apparatus. IR spectra were recorded on a JASCO FT/IR 300E spectrophotometer. UV as well as fluorescence spectra were recorded on a Shimadzu UV-240 spectrophotometer and a JASCO FP-777 spectrofluorometer (Xenon 150 Watt arc lamp; excitation and emission slit band width 5 nm each), respectively. $^1\text{H-NMR}$ spectra were recorded on a Varian GEMINI 200 MHz and MERCURY 300 MHz spectrometers. Mass spectra were recorded on a Finnigan SSQ 7000 (EI 70 eV) spectrometer. The starting compounds **1a–c** (Sharanin et al., 1982; Shestopalov et al., 1984) were prepared according to the reported procedures.

Reaction of **1a–c** with amino acid ester hydrochlorides

A mixture of the appropriate **1a–c** (5 mmol) and the corresponding amino acid ester hydrochloride (10 mmol) in dioxane (20 ml) containing triethylamine (20 mmol) was boiled under reflux for the appropriate time. The separated triethylamine salt was removed by filtration, and the clear reaction mixture was evaporated till dryness under reduced pressure. Then, purified on silica gel TLC to obtain the corresponding **2**, **3**, **4** and **5**.

N-[(4,6-Diphenyl-3-pyridinecarbonitrile)-2-yl]glycine methyl ester (**2a**)

Reaction time 70 h, colourless crystals purified by silica gel TLC using chloroform-light petroleum (60–80°C) as 1:1 v/v for elution, M.p. 144–146°C, yield 70%. – IR: $\nu = 3423$ (NH), 2204 ($\text{C}\equiv\text{N}$), 1739 (CO), 1587, 1554 cm^{-1} ($\text{C}=\text{N}$, $\text{C}=\text{C}$). – $^1\text{H-NMR}$ (CDCl_3): $\delta = 3.81$ (s, 3H, OCH_3), 4.41 (d, $J = 5.7$ Hz, 2H, NCH_2), 5.89 (t, $J = 5.4$ Hz, 1H, NH), 7.23 (s, 1H, pyridine H-5), 7.44–8.05 (m, 10H, arom. H). – MS: m/z (%) = 343 (9) [M], 284 (38), 255 (9). $\text{C}_{21}\text{H}_{17}\text{N}_3\text{O}_2$ (343.37): calcd. C 73.45, H 4.99, N 12.24; found C 73.29, H 4.86, N 12.12.

N-[(4,6-Diphenyl-3-pyridinecarbonitrile)-2-yl]glycine ethyl ester (**2b**)

Reaction time 75 h, colourless crystals purified by silica gel TLC using chloroform-light petroleum (60–80°C) as 2:1 v/v for elution, M.p. 166–168°C, yield 73%. – IR: $\nu = 3413$ (NH), 2204 ($\text{C}\equiv\text{N}$), 1745 (CO), 1587, 1556 cm^{-1} ($\text{C}=\text{N}$, $\text{C}=\text{C}$). – $^1\text{H-NMR}$ (CDCl_3): $\delta = 1.31$ (t, $J = 7.2$ Hz, 3H, CH_3), 4.27 (q, $J = 7.2$ Hz, 2H, OCH_2), 4.38 (d, $J = 5.7$ Hz, 2H, NCH_2), 5.90 (t, $J = 5.4$ Hz, 1H, NH), 7.22 (s, 1H, pyridine H-5), 7.43–8.06 (m, 10H, arom. H). – MS: m/z (%) = 357 (21) [M], 284 (100), 255 (17). $\text{C}_{22}\text{H}_{19}\text{N}_3\text{O}_2$ (357.39): calcd. C 73.93, H 5.36, N 11.76; found C 73.89, H 5.34, N 11.81.

N-[[4-(4-Chlorophenyl)-6-phenyl-3-pyridinecarbonitrile]-2-yl]glycine methyl ester (**2c**)

Reaction time 75 h, colourless crystals purified by silica gel TLC using chloroform-light petroleum (60–80°C) as 1:2 v/v for elution, M.p. 139–141°C, yield 58%. – IR: $\nu = 3405$ (NH), 2208 ($\text{C}\equiv\text{N}$), 1747 (CO), 1571, 1550 cm^{-1} ($\text{C}=\text{N}$, $\text{C}=\text{C}$). – $^1\text{H-NMR}$ (CDCl_3): $\delta = 3.80$ (s, 3H, OCH_3), 4.40 (d, $J = 5.7$ Hz, 2H, NCH_2), 5.89 (t, $J = 5.4$ Hz, 1H, NH), 7.18 (s, 1H, pyridine H-5), 7.45–8.01 (m, 9H, arom. H). – MS: m/z (%) = 377 (38) [M], 318 (100), 289 (6). $\text{C}_{21}\text{H}_{16}\text{ClN}_3\text{O}_2$ (377.81): calcd. C 66.76, H 4.27, N 11.12; found C 66.65, H 4.11, N 11.03.

N-[[4-(4-Chlorophenyl)-6-phenyl-3-pyridinecarbonitrile]-2-yl]glycine ethyl ester (**2d**)

Reaction time 50 h, colourless crystals purified by silica gel TLC using chloroform-light petroleum (60–80°C) as 1:1 v/v for elution, M.p. 158–160°C, yield 61%. – IR: $\nu = 3355$ (NH), 2215 ($\text{C}\equiv\text{N}$), 1751 (CO), 1587, 1552 cm^{-1} ($\text{C}=\text{N}$, $\text{C}=\text{C}$). – $^1\text{H-NMR}$ (CDCl_3): $\delta = 1.32$ (t, $J = 7.2$ Hz, 3H, CH_3), 4.27 (q, $J = 7.2$ Hz, 2H, OCH_2), 4.37 (d, $J = 5.6$ Hz, 2H, NCH_2), 5.95 (t, $J = 5.4$ Hz, 1H, NH), 7.18 (s, 1H, pyridine H-5), 7.45–8.07 (m, 9H, arom. H). – MS: m/z (%) = 392 (92) [M + 1], 390 (30), 318 (100), 289 (32). $\text{C}_{22}\text{H}_{18}\text{ClN}_3\text{O}_2$ (391.83): calcd. C 67.43, H 4.63, N 10.72; found C 67.50, H 4.64, N 10.77.

N-[[4-(4-Methoxyphenyl)-6-phenyl-3-pyridinecarbonitrile]-2-yl]glycine ethyl ester (**2e**)

Reaction time 70 h, colourless crystals purified by silica gel TLC using chloroform-light petroleum (60–80°C) as

1:2 v/v for elution, M.p. 114–116°C, yield 67%. – IR: $\nu = 3428$ (NH), 2204 (C \equiv N), 1745 (CO), 1587, 1550 cm⁻¹ (C=N, C=C). – ¹H-NMR (CDCl₃): $\delta = 1.30$ (t, $J = 7.2$ Hz, 3H, CH₃), 3.86 (s, 3H, OCH₃), 4.26 (q, $J = 7.2$ Hz, 2H, OCH₂), 4.36 (d, $J = 5.4$ Hz, 2H, NCH₂), 5.88 (t, $J = 5.4$ Hz, 1H, NH), 7.02 (d, $J = 9$ Hz, 2H, *p*-sub. benzene), 7.19 (s, 1H, pyridine H-5), 7.43–8.05 (m, 7H, arom. H). – MS: m/z (%) = 387 (37) [M], 314 (100), 285 (2). C₂₃H₂₁N₃O₃ (387.42): calcd. C 71.30, H 5.46, N 10.85; found C 71.39, H 5.52, N 10.71.

N-[[4-(4-Chlorophenyl)-6-phenyl-3-pyridinecarbonitrile]-2-yl]alanine methyl ester (**3a**)

Reaction time 50 h, colourless crystals purified by silica gel TLC using chloroform-light petroleum (60–80°C) as 1:2 v/v for elution, M.p. 168–170°C, yield 51%. – IR: $\nu = 3347$ (NH), 2215 (C \equiv N), 1751 (CO), 1571, 1548 cm⁻¹ (C=N, C=C). – ¹H-NMR (CDCl₃): $\delta = 1.62$ (d, $J = 6.9$ Hz, 3H, CHCH₃), 3.77 (s, 3H, OCH₃), 4.88 (pentate, $J = 6.9$ Hz, 1H, NCH), 5.73 (d, $J = 6.3$ Hz, 1H, NH), 7.17 (s, 1H, pyridine H-5), 7.45–8.03 (m, 9H, arom. H). – MS: m/z (%) = 391 (20) [M], 332 (100). C₂₂H₁₈ClN₃O₂ (391.83): calcd. C 67.43, H 4.63, N 10.72; found C 67.31, H 4.54, N 10.85.

N-[[4-(4-Methoxyphenyl)-6-phenyl-3-pyridinecarbonitrile]-2-yl]alanine methyl ester (**3b**)

Reaction time 60 h, colourless crystals purified by silica gel TLC using chloroform-light petroleum (60–80°C) as 1:1 v/v for elution, M.p. 150–152°C, yield 41%. – IR: $\nu = 3367$ (NH), 2206 (C \equiv N), 1739 (CO), 1585, 1546 cm⁻¹ (C=N, C=C). – ¹H-NMR (CDCl₃): $\delta = 1.61$ (d, $J = 7.2$ Hz, 3H, CHCH₃), 3.77 (s, 3H, OCH₃), 3.88 (s, 3H, OCH₃), 4.87 (pentate, $J = 7.2$ Hz, 1H, NCH), 5.70 (d, $J = 6.9$ Hz, 1H, NH), 7.04 (d, $J = 9.0$ Hz, 2H, *p*-sub. benzene), 7.20 (s, 1H, pyridine H-5), 7.44–8.04 (m, 7H, arom. H). – MS: m/z (%) = 387 (27) [M], 328 (100). C₂₃H₂₁N₃O₃ (387.42): calcd. C 71.30, H 5.46, N 10.85; found C 71.20, H 5.45, N 10.77.

N-[[4-(4-Chlorophenyl)-6-phenyl-3-pyridinecarbonitrile]-2-yl]sarcosine ethyl ester (**4a**)

Reaction time 45 h, almost colourless crystals purified by silica gel TLC using chloroform-light petroleum

(60–80°C) as 1:1 v/v for elution, M.p. 158–160°C, yield 41%. – IR: $\nu = 2204$ (C \equiv N), 1743 (CO), 1596, 1567 cm⁻¹ (C=N, C=C). – ¹H-NMR (CDCl₃): $\delta = 1.28$ (t, $J = 7.2$ Hz, 3H, CH₂CH₃), 3.60 (s, 3H, NCH₃), 4.23 (q, $J = 7.2$ Hz, 2H, OCH₂), 4.35 (s, 2H, NCH₂), 7.19 (s, 1H, pyridine H-5), 7.43–8.01 (m, 9H, arom. H). – MS: m/z (%) = 405 (25) [M], 332 (100). C₂₃H₂₀ClN₃O₂ (405.86): calcd. C 68.06, H 4.97, N 10.35; found C 68.11, H 5.01, N 10.44.

N-[[4-(4-Methoxyphenyl)-6-phenyl-3-pyridinecarbonitrile]-2-yl]sarcosine ethyl ester (**4b**)

Reaction time 50 h, almost colourless crystals purified by silica gel TLC using chloroform-light petroleum (60–80°C) as 1:1 v/v for elution, M.p. 175–177°C, yield 75%. – IR: $\nu = 2200$ (C \equiv N), 1743 (CO), 1608, 1567 cm⁻¹ (C=N, C=C). – ¹H-NMR (CDCl₃): $\delta = 1.28$ (t, $J = 7.2$ Hz, 3H, CH₂CH₃), 3.60 (s, 3H, NCH₃), 3.87 (s, 3H, OCH₃), 4.23 (q, $J = 7.2$ Hz, 2H, OCH₂), 4.34 (s, 2H, NCH₂), 7.03 (d, $J = 9.0$ Hz, 2H, *p*-sub. benzene), 7.23 (s, 1H, pyridine H-5), 7.42–8.02 (m, 7H, arom. H). – MS: m/z (%) = 401 (76) [M], 400 (100), 328 (97). C₂₄H₂₃N₃O₃ (401.44): calcd. C 71.80, H 5.77, N 10.47; found C 71.93, H 5.82, N 10.55.

N-[[4-(4-Chlorophenyl)-6-phenyl-3-pyridinecarbonitrile]-2-yl]-*N'*-glycylglycine ethyl ester (**5a**)

Reaction time 60 h, colourless crystals purified by silica gel TLC using chloroform-light petroleum (60–80°C) as 1:1 v/v for elution, M.p. 200–202°C, yield 67%. – IR: $\nu = 3405$, 3274 (NH), 2206 (C \equiv N), 1754 (CO ester), 1662 (CO amidic), 1577, 1552 cm⁻¹ (C=N, C=C). – ¹H-NMR (CDCl₃): $\delta = 1.17$ (t, $J = 7.2$ Hz, 3H, CH₃), 4.01 (d, $J = 5.1$ Hz, 2H, NCH₂), 4.10 (q, $J = 7.2$ Hz, 2H, OCH₂), 4.30 (d, $J = 4.5$ Hz, 2H, NCH₂), 5.95 (br. s, 1H, NH), 6.60 (br. s, 1H, NH), 7.14 (s, 1H, pyridine H-5), 7.38–7.98 (m, 9H, arom. H). – MS: m/z (%) = 448 (28) [M], 318 (100), 289 (7). C₂₄H₂₁ClN₄O₃ (448.89): calcd. C 64.21, H 4.72, N 12.48; found C 64.10, H 4.67, N 12.52.

N-[[4-(4-Methoxyphenyl)-6-phenyl-3-pyridinecarbonitrile]-2-yl]-*N'*-glycylglycine ethyl ester (**5b**)

Reaction time 60 h, colourless crystals purified by silica gel TLC using chloroform-light petroleum (60–80°C) as

2:1 v/v for elution, M.p. 163–165°C, yield 59%. – IR: $\nu = 3197, 3099$ (NH), 2262 (C \equiv N), 1675 (broad CO), 1619, 1488 cm⁻¹ (C=N, C=C). – ¹H-NMR (CDCl₃): $\delta = 1.22$ (t, $J = 7.2$ Hz, 3H, CH₂CH₃), 3.87 (s, 3H, OCH₃), 4.07 (d, $J = 5.4$ Hz, 2H, NCH₂), 4.15 (q, $J = 7.2$ Hz, 2H, OCH₂), 4.35 (d, $J = 5.4$ Hz, 2H, NCH₂), 6.01 (t, $J = 5.4$ Hz, 1H, NH), 6.83 (t, $J = 5.1$ Hz, 1H, NH), 7.02 (d, $J = 8.4$ Hz, 2H, *p*-sub. benzene), 7.23 (s, 1H, pyridine H-5), 7.44–8.05 (m, 7H, arom. H). – MS: m/z (%) = 445 (78) [M + 1], 444 (68) [M], 314 (100), 285 (6). C₂₅H₂₄N₄O₄ (444.47): calcd. C 67.55, H 5.44, N 12.61; found C 67.58, H 5.44, N 12.63.

2-Amino-4,6-diaryl-3-pyridinecarbonitriles 8a,b

A solution of the appropriate **6** (10 mmol) in water (5 ml) was added to a solution of the corresponding **1** (5 mmol) in pyridine (20 ml). The reaction mixture was boiled under reflux for the appropriate time. The solid separated upon pouring the reaction mixture into ice-cold water (200 ml) and acidification with dil. HCl (5%), was collected, washed with water and purified on silica gel TLC to give the corresponding **8a,b**.

2-Amino-4,6-diphenyl-3-pyridinecarbonitrile (8a)

Reaction time 30 h (for the reaction of **1a** with **6a**), colourless crystals purified by silica gel TLC using chloroform-light petroleum (60–80°C) as 9:1 v/v for elution, M.p. 189–190°C [lit. 184°C (Schmidt, 1965)], yield 37%. – IR: $\nu = 3463, 3301, 3180$ (NH₂), 2206 (C \equiv N), 1637, 1573 cm⁻¹ (C=N, C=C). – ¹H-NMR (CDCl₃): $\delta = 5.36$ (s, 2H, NH₂), 7.21 (s, 1H, pyridine H-5), 7.45–8.02 (m, 10H, arom. H). – MS: m/z (%) = 271 (100) [M], 270 (33), 244 (6). C₁₈H₁₃N₃ (271.30): calcd. C 79.68, H 4.83, N 15.49; found C 79.72, H 4.83, N 15.41.

2-Amino-4-(4-methoxyphenyl)-6-phenyl-3-pyridinecarbonitrile (8b)

Reaction time 45, 50 h (for the reaction of **1c** with **6a** and **6b**, respectively), colourless crystals purified by silica gel TLC using chloroform-light petroleum (60–80°C) as 1:1 v/v for elution, M.p. 182–184°C [lit. 181°C (Sammour et al., 1970)], yield 66, 73% (for the reaction of **1c** with **6a** and **6b**, respectively). – IR: $\nu = 3465, 3307, 3183$ (NH₂), 2206 (C \equiv N), 1639, 1610 cm⁻¹ (C=N, C=C). – ¹H-NMR (CDCl₃): $\delta = 3.88$ (s, 3H, OCH₃), 5.32 (s, 2H, NH₂), 7.04 (d, $J = 8.7$ Hz, 2H, *p*-sub. benzene), 7.19 (s, 1H, pyridine H-5), 7.45–8.01 (m, 7H, arom. H). – MS: m/z (%) = 301

(100) [M], 300 (17), 274 (1). C₁₉H₁₅N₃O (301.33): calcd. C 75.73, H 5.02, N 13.94; found C 75.70, H 4.99, N 13.89.

Acknowledgment

Thanks are due to Dr. Nada N. Nawar, Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University, for allowing the performance of antimicrobial activity screening.

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Authors' address: Adel S. Girgis, Pesticide Chemistry Department, National Research Centre, Dokki, Cairo, Egypt,
Fax: 00202-3370931, E-mail: girgisas10@yahoo.com