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Pseudopeptides based on nicotinic acid with 4-amidoxime unit

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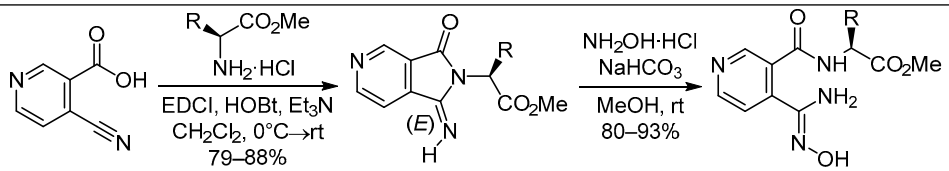


The carbodiimide-mediated coupling of 4-cyanonicotinic acid with L-amino acid methyl esters takes place with the predominant formation of bicyclic compounds – methyl 2-(1-imino-3-oxo-1,3-dihydro-2*H*-pyrrolo[3,4-*c*]pyridin-2-yl)alkanoates, which are monoimino derivatives of 4-azaphthalimide. Under the action of hydroxylamine they undergo pyrrolidine cycle opening with the formation of pseudopeptides – coupling products of nicotinic acid with amino acid esters bearing an amidoxime function at position 4.

Keywords: amidoximes, 4-cyanonicotinic acid, methyl 2-(1-imino-3-oxo-1,3-dihydro-2*H*-pyrrolo[3,4-*c*]pyridin-2-yl)alkanoates, monoimino derivatives of pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-dione, pseudopeptides, pyrrolidine ring opening.

Amidoximes serve as amidine precursors due to their ability to be reduced by mARC-containing enzyme system to the amidines,[1] which are effective mimetics of the guanidine moiety of arginine in the development of anticoagulant drugs. Substitution of amidine group with amidoxime group improves oral bioavailability and has allowed the development of an "amidoximes instead of amidines" prodrug principle.[2] Amidoximes can also be oxidized *in vivo* to serve as donors of nitric oxide (NO)[3,4] that plays a crucial role in numerous physiological and pathological processes.[5] Peptidomimetics with a benzamidine, as well as a benzamidoxime moiety have recently been discovered as potent and selective noncovalent furin inhibitors for the treatment of SARS CoV-2 virus infection.[6] Recently, luminescence properties were found in the solid state of mixed-ligand Zn(II) acetate complexes with pyridine-4-amidoxime ligands.[7]

In continuation of our research on heterocyclic peptidomimetics possessing nonpeptidic β-turn structures, we herein report an efficient synthesis of novel pseudopeptides based on nicotinic acid that are positional isomers of the previously studied pseudopeptides, containing an amidoxime group at position 4 instead of position 2 of the pyridine nucleus.[8]

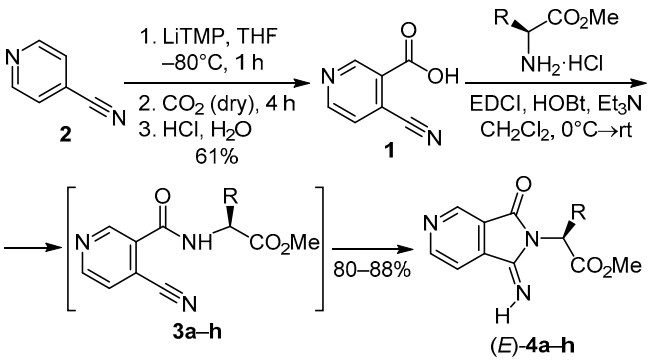
The synthetic route to the target compounds can be similar to those previously elaborated.[8,9] The scheme consisted of sequential coupling of 2-cyanonicotinic acid with esters of α-amino acids and subsequent transformation, by reaction with hydroxylamine, into amidoximes as individual derivatives of intermediate methyl esters of (2*S*)-*N*-[(2-cyanopyridin-3-yl)carbonyl]amino acids or cyclic tautomeric methyl esters of (2*S*)-2-(7-imino-5-oxo-5,7-dihydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl)alkanoic acids, and their mixtures. In this work, 4-cyanonicotinic acid was coupled with amino acid esters using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) as the coupling reagent. Obtained pseudopeptides were then treated with hydroxylamine.

According to the proposed route, the first task was to synthesize the starting 4-cyanonicotinic acid (**1**) (Scheme 1). An attempt was made to synthesize it by the method used for the 2-cyano isomer in our previous work,[8] starting from

the cinchomeronic anhydride through 4-carbamoylnicotinic acid (17% yield), which was transformed by interaction with methyl chloroformate into methyl 4-cyanopyridine-3-carboxylate (9% yield) and then into acid **1** by a saponification. However, our efforts to select and to modify the reaction conditions did not allow to improve the low yields.

Instead, we succeeded in synthesizing 4-cyanonicotinic acid (**1**) by ortho-lithiation of isonicotinonitrile (**2**) using lithium 2,2,6,6-tetramethylpiperidine (LiTMP) followed by carboxylation with dry ice according to modified literature procedure[10] (Scheme 1). Based on the experimental results, we found that only a slight excess of LiTMP (1.15 equiv) was necessary to improve the yield of acid **1** in contrast to 2 equiv used in the original procedure. Carboxylation was carried out by pouring the reaction mixture on dry ice under Ar with vigorous stirring, which was also found more effective than the reverse addition of the electrophile to the lithiated isonicotinonitrile (**2**). The modification of the synthetic procedure allowed to obtain 4-cyanonicotinic acid (**1**) with an improved yield of 61%, which is higher compared to the 41% of the original procedure.

Scheme 1



a R = H, **b** R = Me, **c** R = *i*-Pr, **d** R = *i*-Bu, **e** R = Bn, **f** R = (1*H*-indol-3-yl)methyl, **g** R = CH_2CO_2Me, **h** R = $(CH_2)_2CO_2Me$

Subsequent coupling of 4-cyanonicotinic acid (**1**) with methyl esters of a number of L-α-amino acids (Gly, Ala, Val, Leu, Phe, Trp, Asp, Glu, Pro) was carried out using EDCI as the coupling agent in the presence of 1-hydroxybenzotriazole hydrate (HOBt).[8,9] The cyclization of cyanonicotinamides **3a**–**h** occurred *in situ*. The total yield of amino acid derivatives **4a**–**h** after purification by column chromatography was 80–88% (Table 1).

It was interesting to investigate how fast the coupling of 4-cyanonicotinic acid (**1**) proceeds and whether the basicity of tertiary amine used affects its final result. For a comparative experiment, a weaker base *N*-methylmorpholine (NMM) (pK_a 7.38) was chosen instead of Et_3N (pK_a 10.75), in the presence of which the cyclization rate of compounds **3a**–**h** should not be fast.[11] Thus, in the reaction of acid **1** with the aspartic acid dimethyl ester using NMM, the primary product with *m/z* 292 $[M+H]^+$ (LC/MS), which apparently corresponded to cyanonicotinamide **3g** (t_R 2.756 min), transformed into pyrrolopyridine **4g** (t_R 2.878 min coincided with that of the individual product) much more slowly in the first hours of coupling compared to the reaction when Et_3N was used (Table 2). At the same time, when using NMM, the conversion of acid **1** into cyanonicotinamide **3g** occurred faster. As shown by LC/MC results in both cases, the cyclization of amide **3g** to compound **4g** was completed in about 3 days.

Table 1. NH proton chemical shifts and yields of compounds **4a–h**

Ester	R (Amino acid residue)	NH,* δ, ppm	Yield, %**
4a	H (Gly)	10.52 (*E*) 9.92 (*Z*)	80
4b	Me (Ala)	10.67	81
4c	*i*-Pr (Val)	10.73	80
4d	*i*-Bu (Leu)	10.70	85
4e	Bn (Phe)	10.64	81
4f	(1*H*-Indol-3-yl)methyl (Trp)	10.71	82
4g	CH_2CO_2Me (Asp)	10.72	85
4h	$(CH_2)_2CO_2Me$ (Glu)	10.68	88

* From 600 MHz ^{1}H NMR spectrum in DMSO-d_6.
** Yield after flash-chromatographic separation.

Along with the absorption bands of the two C=O groups in the regions 1752–1736 and 1732–1711 cm^{-1}, in IR spectra of compounds **4a**–**h**, C=N stretching vibrations appear at the region 1667–1658 cm^{-1}, and a sharp strong NH band appears at 3408–3209 cm^{-1}. An absorption band of the cyano group at the 2200 cm^{-1} region which might belong to open form **3** is not observed in contrast to the derivatives of 2-cyanonicotinic acid.[8]

In comparison to the previously studied amino acid derivatives of 2-cyanonicotinic acid,[8] for which the bicyclic tautomeric form based on dihydro-6*H*-pyrrolo[3,4-*b*]pyridine has been dominant along with acyclic 2-cyano-substituted amides, coupling products **4a**–**h** have predominant cyclic tautomeric form of methyl 2-(1-imino-3-oxo-1,3-dihydro-2*H*-pyrrolo[3,4-*c*]pyridin-2-yl)alkanoates. Characteristic signal of the C=NH imino group proton at the region of 10 ppm in the ^{1}H NMR spectra supports this hypothesis (Table 1). As follows from ^{1}H NMR spectra of compounds **4a**–**h** (see Supplementary information file), they adopt the *E*-configuration at imino C=N bond when NH proton is orientated toward the aromatic ring in a similar manner to what was observed previously for isomeric compounds.[8]

Thus, the structure of pyrrolopyridine **4d** was confirmed using the following NMR experiments (DMSO-d_6): ^{1}H, ^{13}C-APT, COSY, ^{1}H–^{13}C HSQC, ^{1}H–^{13}C HMBC, and NOESY

Table 2. The ratio of acid **1** to cyanocarboxamide **3g** and pyrrolopyridine **4g** in reaction mixtures*

Time, h	Ratio of compounds **1**:**3g**:**4g**	
	NMM	Et_3N
0.5	39:57:4	39:46:15
1	22:73:5	38:36:26
1.5	9:84:7	26:44:30
2	5:87:8	18:36:46
2.5	2:89:9	13:27:60
2 days	0:9:91	0:5:95
3 days	100	100

* LC/MC results of the reaction mixture.

experiments (see Supplementary information file). It should be noted that the NOE correlation between the NH proton and the aromatic H-5 Py proton (position 5 of the pyridine ring) at 8.17 ppm is relatively strong (3%), indicating significant spatial proximity of these protons in the molecule.

Furthermore, it is interesting to mention the differences observed in the ^{1}H NMR spectra of compound **4d** when measured in DMSO-d_6 and $CDCl_3$. Both NH and H-5 Py being in proximity exhibit significant broadening in $CDCl_3$ compared to DMSO-d_6. This phenomenon can be attributed to the poor solvation of NH group in $CDCl_3$ as well as the slight acidity of $CDCl_3$. These factors can induce slow (in the NMR time scale) exchange processes involving the NH group, leading to its broadening, and consequently, the broadening of the H-5 Py proton peak at 7.66 ppm due to its spatial proximity.

The analysis of the ^{1}H NMR spectra of pyrrolopyridine **4a** in DMSO-d_6 shows doubling of all proton signals, and most importantly of the NH proton signal, due to the probable occurrence of a mixture of *Z*- and *E*-isomers in the ratio of approximately 15:85. Prior to conducting the NOESY experiment on substrate **4a** mixture illustrated in Figure 1, we have performed a temperature-dependent ^{1}H NMR at 80°C in DMSO-d_6 (see Supplementary information file). The results indicated that the doubling of all signals in the ^{1}H NMR spectrum occurs neither due to rotamery issues of the aliphatic substituent nor slow (on the NMR time scale) *E*/*Z* isomerization at C=N bond.

The NOESY experiment proved (see Supplementary information file) to be highly beneficial in distinguishing the *E*- and *Z*-isomers of molecule **4a**, as all relevant signals were well resolved in the ^{1}H NMR spectrum (Fig. 1). For the *E*-isomer, a strong NOE was observed between the pyridine H-5 Py at 8.19 ppm and NH at 10.52 ppm. Conversely, for the *Z*-isomer, an NOE was observed between the aliphatic CH_2 group at 4.65 ppm and NH at 9.92 ppm. In the ^{1}H NMR spectrum of compound **4a**, a high-field shift of the NH and H-5 Py signals of the *Z*-isomer was observed relative to the signals of the corresponding protons of the *E*-isomer. The signals of the aliphatic fragment, on the contrary, were shifted to the low field in *Z*-isomer.

In the ^{13}C NMR spectrum (APT) of compound **4a**, a noticeable difference between *E*- and *Z*-isomers was also observed in the position of the carbon atom signals of the amidine fragment N=C–N (157.5 ppm (*E*) *vs*. 153.4 ppm (*Z*)), as well as signals of C-4 Py atom (139.0 ppm (*E*) *vs*. 143.9 ppm (*Z*)) and C-5 Py (117.2 ppm (*Z*) *vs*. 117.9 ppm (*E*)).

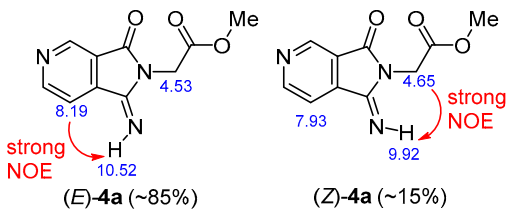


Figure 1. Structures of the *E*- and *Z*-isomers of compound **4a**, confirmed by NOESY experiment (DMSO-d_6). ^{1}H NMR chemical shifts are shown in ppm.

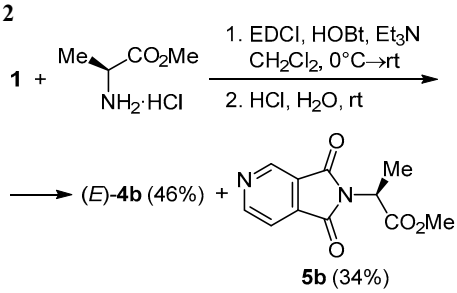
Slightly noticeable signals of the corresponding minor *Z*-isomers occurred in the same region in the ^{1}H NMR spectra of other synthesized acylamidines **4b**–**h** at a level not exceeding 3%. This is possibly due to greater steric hindrances in the formation of *Z*-isomers affecting the cyclization of the corresponding cyanocarboxamides **3b**–**h**.

^{1}H NMR spectra of pyrrolopyridines **4a**–**h** in $CDCl_3$ showed only one set of signals. The NH signal appeared as a broadened singlet at about 9 ppm, high-field shifted by more than 1.5 ppm compared to the corresponding signal recorded in DMSO-d_6. In addition, the H-5 signal had the shape of a broadened doublet or a broadened singlet of reduced intensity. In the ^{13}C NMR spectra in $CDCl_3$, the signals of C-4 Py and C-5 Py atoms during prolonged accumulation of the spectrum have been detected as barely noticeable broadened singlets of low intensity. This may be due to the dynamic processes in the molecules of compounds **4a**–**h** occurring in $CDCl_3$ solution.

Compounds **4a**–**h** are the corresponding monoimino derivatives of pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-dione that have not yet been reported. The most studied are the 4-aza analogs of phthalimide, and cinchomeronic acid anhydride interaction with various amines is used in the synthesis of *N*-substituted 1*H*-pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-diones.[12] A milder procedure is the amination of methyl 3-chlorocarbonylisonicotinate, that was prepared from cinchomeronic acid obtained by conversion of the starting anhydride.[13] *N*-Benzyl-substituted pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-diones have raised a particular interest as a new class of antimycobacterial substances.[14,15]

N-Substituted 1*H*-pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-dione derivatives have been obtained as byproducts of synthesis of the corresponding imines derived from the hydrolysis of the imino group. Thus, during the isolation of the target reaction products, in particular, acylamidine **4b**, a standard washing of the reaction mixture with 0.1 M HCl was used.[8,9] However, even short-term acid exposure reduced the yield of the target compound **4b** due to the hydrolysis of the exocyclic imino group with the formation of dioxopyrrolopyridine **5b** (Scheme 2). Compound **5b** was isolated by flash chromatography, and its structure was confirmed by LC/MS, HRMS, NMR, and IR spectroscopy.

Scheme 2

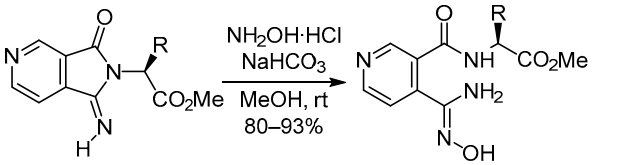


It should be noted that the formation of hydrolysis products similar to compound **5b** was observed even during the LC/MS analysis of purified acylamidines **4a**–**h**, which is probably due to the use of a mobile phase consisting of formic acid (0.1%, v/v) *via* gradient elution. Another reason for the decrease in the yield of compound **4b** during

CH_2Cl_2 extraction was its partial solubility in water. Therefore, brine was subsequently used to wash the reaction mixture.

The dihydropyrroline ring of methyl (*E*)-2-(1-imino-3-oxo-1,3-dihydro-2*H*-pyrrolo[3,4-*c*]pyridin-2-yl)alkanoates **4a**–**h** under the action of hydroxylamine undergoes ring opening to give methyl esters of *N*-{4-[amino(hydroxyimino)methyl]pyridin-3-yl}carbonyl-substituted α-amino acids **6a**–**h** as observed for similar previously studied compounds (Scheme 3).[8,9] However, the yields of compounds **6a**–**h** using the previously described method were quite low and did not exceed 50%. Their noticeable solubility in water along with other reasons contributed to the loss of the products during the isolation. The replacement of Et_3N with $NaHCO_3$ to generate free NH_2OH *in situ* and avoiding the use of water during the isolation of the target compounds made it possible to increase their yields to more than 80%.

Scheme 3



(*E*)-**4a–h** **6a–h**

a R = H, **b** R = Me, **c** R = *i*-Pr, **d** R = *i*-Bu, **e** R = Bn, **f** R = (1*H*-indol-3-yl)methyl, **g** R = CH_2CO_2Me, **h** R = $(CH_2)_2CO_2Me$

As in the case of similar isoindolones,[9] the formation of an undesirable but expected side product with *m*/*z* 164 $[M+H]^+$, which undoubtedly assigned as 1-(hydroxyimino)-1,2-dihydro-3*H*-pyrrolo[3,4-*c*]pyridin-3-one, was observed. The content of the latter increased with an extension of time of keeping the reaction mixtures without separation, as well as with even a slight heating, which led to the decrease of the yields of open-chain compounds **6a**–**h**. Therefore, it was necessary to work up the reaction mixture no later than 16 h after the start of the reaction, and to carry out all solvent evaporation at a temperature not higher than 40°C.

In ^{1}H NMR spectra of compounds **6a**–**h**, characteristic two-proton signals are observed approximately at 5.8 ppm and intense single-proton signals at ~9.9 ppm which correspond to NH_2 and OH protons, respectively, of *N*'-hydroxycarbamimidoyl group. In the ^{13}C NMR spectra of compounds **6a**–**h**, the carbon signal of the amidoxime group located approximately at 150.6 ppm and only in the case of proline derivative **6i** is shifted to high field (149.6 ppm), i.e., in general, this signal is shifted to high field by about 7 ppm in comparison to cyclic acylamidines **4a**–**h**. It is also worth to note the shift of the H-5 Py signal to the high field by approximately 0.6 ppm associated with the *Z*-configuration of the C=N–OH double bond. In the ^{1}H NMR spectrum of amidoxime **6d** in $CDCl_3$, the OH signal is not observed, probably due to rapid proton exchange. The position of the CONH group signal at 7.86 ppm should also be noted, which is shifted by almost 1 ppm to high field compared to this signal in DMSO-d_6 solution.

Single crystals of valine-derived amidoxime **6c** (R = *i*-Pr) suitable for X-ray diffraction analysis have been grown from saturated $CHCl_3$–MeOH, 9:1 solution (Fig. 2). Compound **6c** crystallizes in the chiral space group $P2_1$ that confirms the existence of a pure enantiomer in the crystal phase. The absence of a heavy atom makes it impossible to determine the absolute configuration of the asymmetric center C(8).

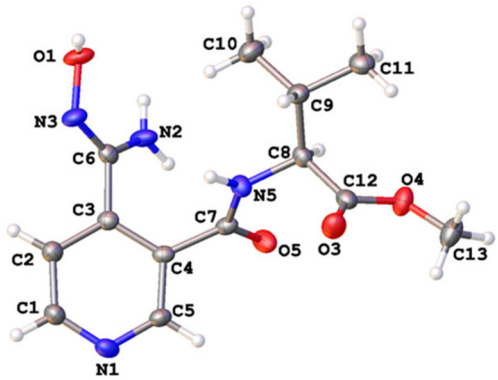


Figure 2. Molecular structure of methyl (*Z*)-[4-(*N*'-hydroxycarbamimidoyl)nicotinoyl]-L-valinate (**6c**) with the atom numbering scheme. Thermal ellipsoids are shown at 50% probability level.

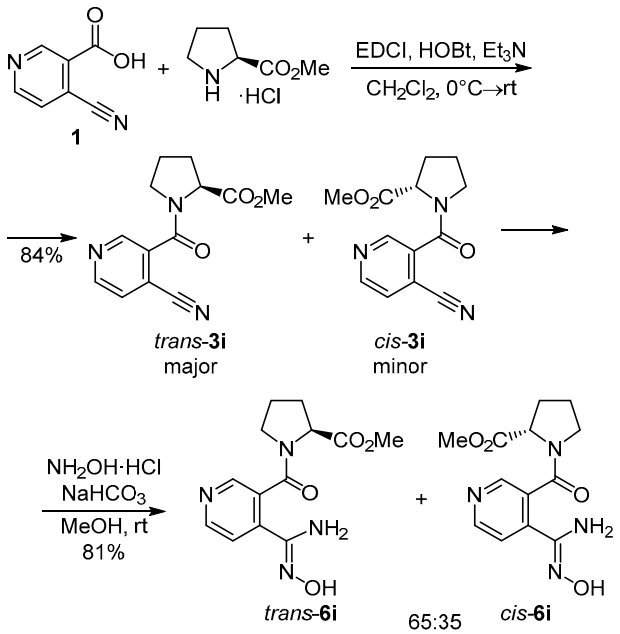
Two substituents of the pyridine ring are located in a vicinal position, which causes a significant steric repulsion between them. This repulsion is compensated by twisting of the exocyclic C(6)–C(3)–C(4)–C(7) angle up to 10.3(6)° and significant rotation of both substituents with respect to the aromatic cycle: C(5)–C(4)–C(7)–O(5) and C(4)–C(3)–C(6)–N(2) torsion angles are 62.3(6)° and 45.1(6)°, respectively. The C(4)–C(7) and C(3)–C(6) bond lengths (1.491(6) and 1.486(6) Å) also confirm some disturbance of conjugation between the π-systems of the carbamide fragment or the amino(hydroxyimino)methyl group and the aromatic cycle. In the crystal phase molecules **6c** form layers parallel to the (100) crystallographic plane due to the formation of the N–H···O, N–H···N, and O–H···O intermolecular hydrogen bonds (Table 3).

Unlike other amino acids, in the case of proline, the formation of only a noncyclic product is clearly possible (Scheme 4). The presence of a partially double peptide bond N–CO leads to the appearance of cyanonicotinamide **3i** as a pair of *cis*- and *trans*-isomers, in a ratio of 7:3. Similarly to the previously studied proline derivatives of 2-cyanonicotinic acid,[8] it can be assumed that the major product (≈71%) is the *trans*-isomer, and the minor product is the *cis*-isomer (≈29%). Under similar conditions, a Pro amidoxime **6i** was synthesized from cyano derivative **3i**. Its ^{1}H NMR spectrum reveals doubling of signals, the intensity of which suggests that the ratio of *trans*-/*cis*-isomers is ~65:35.

Table 3. Characteristics of intermolecular hydrogen bonds in the crystal of compound **6c**

Bond	Symmetry operation	H···O/H···N distance, Å	Bond angle, deg.
N(2)–H···O(5')	$1-x, 0.5+y, -z$	2.25(6)	146(6)
N(5)–H···N(1')	$1-x, 0.5+y, 1-z$	2.04(6)	176(5)
O(1)–H···O(5')	$x, 1+y, z$	2.0(1)	162(10)

Scheme 4



In conclusion, the dominant pathway of 4-cyanonicotinic acid coupling with L-amino acid methyl esters is the formation of bicyclic compounds methyl 2-(1-imino-3-oxo-1,3-dihydro-2*H*-pyrrolo[3,4-*c*]pyridin-2-yl)alkanoates. Under the action of hydroxylamine they undergo the pyrrolidine cycle opening with the formation of the nicotinic acid based pseudopeptides with amidoxime function at position 4. The obtained substances can serve as precursors in the synthesis of pseudopeptides imitating β-turn by further modification of both the amino acid residue and the amidoxime group. The obtained pyridine-based amidoximes could serve as ligands for the elaboration of the metal complexes with luminescence properties.

Experimental

IR spectra have been registered on a PerkinElmer Spectrum BX FT-IR spectrometer for samples prepared as KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance DRX-400 (400 and 100 MHz, respectively), Bruker Avance DRX-500 (500 and 125 MHz, respectively), and Bruker Avance DRX-600 (600 and 150 MHz, respectively) spectrometers. TMS was used as internal standard. HPLC/MS have been recorded on an Agilent Technologies 1260 Infinity II Quaternary system (electrospray ionization at atmospheric pressure) equipped with an Infinity Lab Poroshell 120EC-C18 column (4.6 × 100 mm, 2.7 μm), solvent MeOH, gradient elution with 0.1% aqueous HCOOH in H_2O with increasing admixture of 0.1% HCOOH in MeOH. High-resolution mass spectra were recorded on an Agilent 6224 TOF LC/MS. Melting points have been determined using an OptiMelt automated melting point system. Flash column chromatography was carried out using silica gel 60, 40–63 μm.

All chemicals have been purchased from Enamine Ltd.

4-Cyanonicotinic acid (1). *n*-BuLi (44 ml, 0.110 mol, 2.5 M) was added slowly at –30°C to a stirred solution of 2,2,6,6-tetramethylpiperidine (16.25 g, 0.115 mol) in THF (100 ml). The obtained mixture was stirred for 45 min at this temperature and then was cooled down to –80°C. Isonicotinonitrile (**2**) (10.5 g, 0.10 mol) in THF (50 ml) was added dropwise to the mixture. After 1 h at –80°C, the reaction mixture was poured onto dry ice under Ar and kept for 4 h with vigorous stirring. Then it was evaporated to dryness, and the residue was dissolved in H_2O (100 ml). The resulting solution was acidified to pH 6 using 1 M HCl (230 ml) and extracted with EtOAc (3×150 ml). After evaporation of the solvent, a white powder of 4-cyanonicotinic acid (**1**) was obtained. Yield 9.03 g (61%), white powder, mp 174–176°C. IR spectrum, ν, cm^{-1}: 3468, 2462, 2240 (CN), 1909, 1719 (CO), 1668, 1600 (C=N), 1325, 1281, 1161, 1059, 854. ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 8.01 (1H, d, *J* = 4.9, H-5 Py); 9.00 (1H, d, *J* = 5.1, H-6 Py); 9.25 (1H, s, H-2 Py). ^{13}C NMR spectrum (125 MHz, DMSO-d_6), δ, ppm: 116.8 (CN); 120.9 (C-5 Py); 127.8 (C-3 Py); 128.8 (C-4 Py); 152.5 (C-2 Py); 154.8 (C-6 Py); 165.1 (COO). Found, *m*/*z*: 149.0347 [M+H]$^+$. $C_7H_5N_2O_2$. Calculated, *m*/*z*: 149.0346.

Synthesis of methyl 2-(1-imino-3-oxo-1,3-dihydro-2*H*-pyrrolo[3,4-*c*]pyridin-2-yl)alkanoates 4a–h and 4-cyanonicotinamide 3i (General method). To a suspension of 4-cyanonicotinic acid (**1**) (0.37 g, 2.5 mmol) in CH_2Cl_2 (5 ml), HOBt (0.371 g, 2.75 mmol, 1.1 equiv), an appropriate methyl ester of L-amino acid hydrochloride (2.5 mmol), EDCI (0.502 g, 2.62 mmol, 1.05 equiv), and Et_3N (0.518 g, 5.12 mmol, 2.05 equiv) were added at 0°C. The mixture then was warmed up to room temperature and stirred overnight. Then the resulting clear amber solution was diluted with CH_2Cl_2 (10 ml) and brine (5 ml). The organic layer was separated and washed with brine (5 ml). The combined aqueous layer was further extracted with CH_2Cl_2 (10 ml). The combined organic layer then was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash column chromatography (gradient elution of MTBE–*i*-PrOH, 0:100–20:80) to afford the desired products.

Methyl (*Z*/*E*)-2-(1-imino-3-oxo-1,3-dihydro-2*H*-pyrrolo-[3,4-*c*]pyridin-2-yl)acetate (4a). Yield 0.44 g (80%), pale-yellow powder, mp 144–146°C. IR spectrum, ν, cm^{-1}: 3457, 3289 (N–H), 2958, 1748 (CO), 1732 (CO), 1664 (C=N), 1612, 1434, 1391, 1377, 1333, 1242, 1222, 1134, 946. ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 3.69–3.70 (3H, m, CH_3O (*Z* + *E*)); 4.53 (1.68H, s, CH_2 (*E*)); 4.65 (0.32H, s, CH_2 (*Z*)); 7.93 (0.16H, d, *J* = 4.9, H-5 Py (*Z*)); 8.19 (0.84H, d, *J* = 5.0, H-5 Py (*E*)); 9.03 (0.16H, d, *J* = 4.9, H-6 Py (*Z*)); 9.07 (0.84H, d, *J* = 5.0, H-6 Py (*E*)); 9.08–9.09 (1H, m, H-2 Py (*Z* + *E*)); 9.92 (0.16H, s, NH (*Z*)); 10.52 (0.84H, s, NH (*E*)). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm: 40.1 (2 signals, CH_2 (*Z*), CH_2 (*E*)); 53.2 (CH_3O (*E*)); 53.3 (CH_3O (*Z*)); 117.2 (C-5 Py (*Z*)); 117.9 (C-5 Py (*E*)); 125.6 (C-3 Py (*E*)); 126.2 (C-3 Py (*Z*)); 139.0 (C-4 Py (*E*)); 143.9 (C-4 Py (*Z*)); 145.2 (C-2 Py (*E*)); 145.3 (C-2 Py (*Z*)); 153.4 (N=C–N (*Z*)); 155.7 (C-6 Py (*Z*)); 155.8 (C-6 Py (*E*)); 157.5 (N=C–N (*E*)); 166.7 (O=C–N (*Z*)); 166.9 (O=C–N (*E*)); 169.1 (2 signals, COO (*Z*), COO (*E*)). Found, *m*/*z*: 220.0720 [M+H]$^+$. $C_{10}H_{10}N_3O_3$. Calculated, *m*/*z*: 220.0717.

Methyl (*S*)-2-(1-imino-3-oxo-1,3-dihydro-2*H*-pyrrolo-[3,4-*c*]pyridin-2-yl)propanoate (4b). Yield 0.47 g (81%), pale-yellow powder, mp 135–137°C. IR spectrum, ν, cm^{-1}: 3443, 3288 (N–H), 3002, 1736 (CO), 1719 (CO), 1660 (C=N), 1426, 1415, 1312, 1277, 1262, 1224, 1133, 1091, 872. ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.56 (3H, d, *J* = 107.1, CHC<u>H</u>$_3$); 3.62 (3H, s, CH_3O); 5.16 (1H, q, *J* = 7.2, C<u>H</u>CH_3); 8.18 (1H, d, *J* = 4.9, H-5 Py); 9.07–9.08 (2H, m, H-2,6 Py); 10.67 (1H, s, NH). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm: 15.6 (CH<u>C</u>H_3); 47.9 (<u>C</u>HCH_3); 53.2 (CH_3O); 117.8 (C-5 Py); 125.5 (C-3 Py); 138.9 (C-4 Py); 145.2 (C-2 Py); 155.7 (C-6 Py); 157.2 (N=C–N); 166.7 (O=C–N); 171.2 (COO). Found, *m*/*z*: 234.0878 $[M+H]^+$. $C_{11}H_{12}N_3O_3$. Calculated, *m*/*z*: 234.0873.

Methyl 2-(1,3-dioxo-1,3-dihydro-2*H*-pyrrolo[3,4-*c*]-pyridin-2-yl)propanoate (5b) was chromatographically isolated from the mixture with compound **4b** (yield 0.26 g (46%)) and other products obtained according to the general method, which in this case has included washing the reaction mixture with 0.1 M HCl. Yield 0.20 g (34%), light-beige powder, mp 70–72°C. IR spectrum, ν, cm^{-1}: 3478, 3008, 1783 (CO), 1746 (CO), 1720 (CO), 1611, 1453, 1418, 1383, 1354, 1302, 1255, 1152, 1077, 886. ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.55 (3H, d, *J* = 7.2, CHC<u>H</u>$_3$); 3.65 (3H, s, CH_3O); 5.06 (1H, q, *J* = 7.2, C<u>H</u>CH_3); 7.94 (1H, d, *J* = 4.8, H-5 Py); 9.15 (1H, d, *J* = 4.8, H-6 Py); 9.17 (1H, s, H-2 Py). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm: 15.5 (CH<u>C</u>H_3); 48.0 (<u>C</u>HCH_3); 53.6 (CH_3O); 118.0 (C-5 Py); 126.2 (C-3 Py); 139.8 (C-4 Py); 145.2 (C-2 Py); 157.2 (C-6 Py); 166.8 (O=C–N); 167.2 (O=C–N); 170.7 (COO). Found, *m*/*z*: 235.0715 $[M+H]^+$. $C_{11}H_{11}N_2O_4$. Calculated, *m*/*z*: 235.0713.

Methyl (*S*)-2-(1-imino-3-oxo-1,3-dihydro-2*H*-pyrrolo-[3,4-*c*]pyridin-2-yl)-3-methylbutanoate (4c). Yield 0.52 g (80%), pale-yellow powder, mp 114–116°C. IR spectrum, ν, cm^{-1}: 3383, 3291 (N–H), 3106, 2964, 1740 (CO), 1663 (C=N), 1594, 1436, 1422, 1411, 1359, 1290, 1217, 1094, 896. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 0.77 (3H, d, *J* = 6.8, CH_3); 1.11 (3H, d, *J* = 6.6, CH_3); 2.62–2.71 (1H, m, β-CH); 3.58 (3H, s, CH_3O); 4.71 (1H, d, *J* = 8.7, α-CH); 8.19 (1H, d, *J* = 4.8, H-5 Py); 9.08–9.10 (2H, m, H-2,6 Py); 10.73 (1H, s, NH). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm: 20.0 (CH_3); 22.0 (CH_3); 28.7 (β-CH); 53.0 (CH_3O); 57.7 (α-CH); 118.0 (C-5 Py); 125.2 (C-3 Py); 138.6 (C-4 Py); 145.4 (C-2 Py); 155.8 (C-6 Py); 157.9 (N=C–N); 167.1 (O=C–N); 170.1 (COO). Found, *m*/*z*: 262.1188 $[M+H]^+$. $C_{13}H_{16}N_3O_3$. Calculated, *m*/*z*: 262.1186.

Methyl (*S*)-2-(1-imino-3-oxo-1,3-dihydro-2*H*-pyrrolo-[3,4-*c*]pyridin-2-yl)-4-methylpentanoate (4d). Yield 0.58 g (85%), pale-yellow powder, mp 89–91°C. IR spectrum, ν, cm^{-1}: 3395, 3209 (N–H), 2960, 2871, 1740 (CO), 1660 (C=N), 1617, 1597, 1422, 1255, 1216, 1165, 1080, 866. ^{1}H NMR spectrum (500 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 0.92 (3H, d, *J* = 6.7, CH_3); 0.96 (3H, d, *J* = 6.5, CH_3); 1.44–1.50 (1H, m, γ-CH); 1.97–2.02 (1H, m) and 2.42 (1H, br. s, CH_2); 3.72 (3H, s, CH_3O); 5.18–5.21 (1H, m, α-CH); 7.64 (1H, br. s, H-5 Py); 8.95 (1H, br. s, NH); 9.02 (1H, d, *J* = 4.9, H-6 Py); 9.16 (1H, s, H-2 Py). ^{13}C NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 21.6 (CH_3); 23.5 (CH_3); 25.4 (γ-CH); 37.5 (CH_2); 51.5 (α-CH); 53.0 (CH_3O); 115.4 (br. s, C-5 Py); 125.3 (C-3 Py); 138.6 (br. s, C-4 Py); 145.7 C-2 Py); 154.6 (C-6 Py); 158.9 (br. s, N=C–N); 166.7 (O=C–N); 170.7 (COO). ^{1}H NMR (600 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 0.84 (3H, d, *J* = 6.7, CH_3); 0.89 (3H, d, *J* = 6.5, CH_3); 1.40–1.43 (1H, m, γ-CH); 1.83–1.88 (1H, m) and 2.26–2.31 (1H, m, CH_2); 3.61 (3H, s, CH_3O); 5.09–5.12 (1H, m, α-CH); 8.19 (1H, d, *J* = 4.9, H-5 Py); 9.08 (1H, d, *J* = 4.6, H-6 Py) 9.09 (1H, s, H-2 Py); 10.70 (1H, s, NH). ^{13}C NMR (150 MHz, DMSO-d_6), δ, ppm: 21.6 (CH_3); 23.5 (CH_3); 24.9 (γ-CH); 37.2 (CH_2); 50.5 (α-CH); 52.8 (CH_3O); 117.4 (C-5 Py); 124.8 (C-3 Py); 138.3 (C-4 Py); 144.8 (C-2 Py); 155.3 (C-6 Py); 157.2 (N=C–N); 166.6 (O=C–N); 170.5 (COO). Found, *m*/*z*: 276.1343 $[M+H]^+$. $C_{14}H_{18}N_3O_3$. Calculated, *m*/*z*: 276.1343.

Methyl (*S*)-2-(1-imino-3-oxo-1,3-dihydro-2*H*-pyrrolo-[3,4-*c*]pyridin-2-yl)-3-phenylpropanoate (4e). Yield 0.63 g (81%), cream-colored powder, mp 115–117°C. IR spectrum, ν, cm^{-1}: 3438, 3288 (N–H), 2946, 1752 (CO), 1726 (CO), 1658 (C=N), 1432, 1412, 1218, 1117, 859. ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 3.46–3.49 (2H, m, CH_2); 3.66 (3H, s, CH_3O); 5.39–5.42 (1H, m, α-CH); 7.05–7.17 (5H, m, H Ph); 8.11–8.12 (1H, m, H-5 Py); 9.01 (1H, s, H-2 Py); 9.04 (1H, d, *J* = 5.0, H-6 Py); 10.64 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 34.6 (CH_2); 53.4 (α-CH); 53.7 (CH_3O); 117.8 (C-5 Py); 124.9 (C-3 Py); 127.4 (C-4 Ph); 129.1 (C-3,5 Ph); 129.8 (C-2,6 Ph); 138.1 (C-1 Ph); 138.5 (C-3 Py); 145.2 (C-2 Py); 155.9 (C-6 Py); 157.3 (N=C–N); 166.7 (O=C–N); 170.3 (COO). Found, *m*/*z*: 310.1175 $[M+H]^+$. $C_{17}H_{16}N_3O_3$. Calculated, *m*/*z*: 310.1186.

Methyl (*S*)-2-(1-imino-3-oxo-1,3-dihydro-2*H*-pyrrolo-[3,4-*c*]pyridin-2-yl)-3-(1*H*-indol-3-yl)propanoate (4f). Yield 0.71 g (82%), orange powder, mp 91–93°C. IR spectrum, ν, cm^{-1}: 3408 (N–H), 3200, 2926, 1736 (CO), 1660 (C=N), 1617, 1413, 1270, 1108, 745. ^{1}H NMR spectrum (500 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 3.69–3.96 (5H, m, CH_2, CH_3O); 5.51 (1H, br. s, α-CH); 6.99 (1H, s, H-2 indole); 7.02 (1H, dd, ^2J = 7.7, ^1J = 7.3, H-6 indole); 7.10 (1H, dd, ^2J = 7.3, ^1J = 7.6, H-5 indole); 7.24 (1H, d, *J* = 8.1, H-4 indole); 7.48 (1H, br. s, H-5 Py); 7.58 (1H, d, *J* = 7.9, H-7 indole); 8.05 (1H, s, NH indole); 8.86 (1H, br. s, C=NH); 8.90 (1H, d, *J* = 5.0, H-6 Py); 9.03 (1H, s, H-2 Py). ^{13}C NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 24.8 (CH_2); 53.1 (α-CH); 53.4 (CH_3O); 111.4 (C-3 indole); 111.5 (C-7 indole); 115.3 (C-5 Py); 118.7 (C-4 indole); 119.7 (C-5 indole); 122.3 (C-6 indole); 122.9 (C-2 indole); 125.1 (C-3 Py); 127.5 (C-4a indole); 136.3 (C-7a indole); 138.5 (C-4 Py); 145.5 (C-2 Py); 154.4 (C-6 Py); 158.8 (N=C–N); 166.5 (O=C–N); 170.2 (O–C=O). ^{1}H NMR (600 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 3.55–3.58 (1H, m, CH_2); 3.68 (3H, s, CH_3O); 3.69–3.73 (1H, m, CH_2); 5.39–5.41 (1H, m, α-CH); 6.87 (1H, dd, ^1J = 7.6, ^2J = 7.5, H-5 indole); 6.98 (1H, dd, ^1J = 7.4, ^2J = 7.3, H-6 indole); 7.02 (1H, d, *J* = 2.4, H-2 indole); 7.25 (1H, d, *J* = 8.1, H-7 indole); 7.48 (1H, d, *J* = 7.9, H-4 indole); 8.11 (1H, d, *J* = 4.2, H-5 Py); 8.99 (1H, s,

H-2 Py); 9.03 (1H, d, *J* = 5.0, H-6 Py); 10.62 (1H, s, C=NH); 10.71 (1H, s, NH indole). ^{13}C NMR (150 MHz, DMSO-d_6), δ, ppm: 24.3 (CH_2); 52.9 (α-CH); 53.0 (CH_3O); 110.1 (C-3 indole); 111.8 (C-7 indole); 117.3 (C-5 Py); 118.4 (C-4 indole); 118.7 (C-5 indole); 121.3 (C-6 indole); 123.9 (C-2 indole); 124.7 (C-3 Py); 127.6 (C-4a indole); 136.4 (C-7a indole); 138.2 (C-4 Py); 144.7 (C-2 Py); 155.3 (C-6 Py); 157.0 (N–C=N); 166.4 (N–C=O); 170.1 (O–C=O). Found, *m*/*z*: 349.1292 $[M+H]^+$. $C_{19}H_{17}N_4O_3$. Calculated, *m*/*z*: 349.1295.

Dimethyl (*S*)-2-(1-imino-3-oxo-1,3-dihydro-2*H*-pyrrolo-[3,4-*c*]pyridin-2-yl)succinate (4g). Yield 0.62 g (85%), cream-colored powder, mp 103–105°C. IR spectrum, ν, cm^{-1}: 3410, 3287 (N–H), 2957, 1738 (CO), 1711 (CO), 1667 (C=N), 1609, 1415, 1376, 1311, 1232, 1172, 1134, 873. ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 3.03–3.08 (1H, m) and 3.25–3.28 (1H, m, CH_2); 3.59 (3H, s, CH_3O); 3.63 (3H, s, CH_3O); 5.47–5.50 (1H, m, α-CH); 8.18 (1H, d, *J* = 5.0, H-5 Py); 9.09 (1H, d, *J* = 5.0, H-6 Py); 9.11 (1H, s, H-2 Py); 10.72 (1H, s, NH). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm: 34.3 (CH_2); 48.7 (α-CH); 52.7 (CH_3O); 53.7 (CH_3O); 117.9 (C-5 Py); 125.3 (C-3 Py); 138.8 (C-4 Py); 145.4 (C-2 Py); 155.9 (C-6 Py); 157.1 (N–C=N); 166.6 (N–C=N); 170.0 (α-COO); 171.3 (β-COO). Found, *m*/*z*: 292.0931 $[M+H]^+$. $C_{13}H_{14}N_3O_5$. Calculated, *m*/*z*: 292.0928.

Dimethyl (*S*)-2-(1-imino-3-oxo-1,3-dihydro-2*H*-pyrrolo-[3,4-*c*]pyridin-2-yl)pentanedioate (4h). Yield 0.67 g (88%), cream-colored crystalline powder, mp 72–74°C. IR spectrum, ν, cm^{-1}: 3455, 3295 (N–H), 2957, 1743 (CO), 1664 (C=N), 1610, 1410, 1277, 1176, 1115, 850. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 2.34–2.40 (4H, m, $2CH_2$); 3.48 (3H, s, CH_3O); 3.62 (3H, s, CH_3O); 5.09–5.12 (1H, m, α-CH); 8.16–8.19 (1H, m, H-5 Py); 9.06–9.08 (2H, m, H-2,6 Py); 10.68 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 24.5 (β-CH_2); 30.8 (γ-CH_2); 51.7 (α-CH); 52.2 (CH_3O); 53.4 (CH_3O); 117.9 (C-5 Py); 125.4 (C-3 Py); 138.9 (C-4 Py); 145.3 (C-2 Py); 155.7 (C-6 Py); 157.6 (N=C–N); 167.1 (O=C–N); 170.5 (α-COO); 173.6 (γ-COO). Found, *m*/*z*: 328.0907 $[M+Na]^+$. $C_{14}H_{15}N_3NaO_5$. Calculated, *m*/*z*: 328.0904.

Methyl *cis*/*trans*-(4-cyanonicotinoyl)-L-prolinate (3i). Yield 0.54 g (84%), beige crystalline powder, mp 116–118°C. IR spectrum, ν, cm^{-1}: 3455, 2955, 2238 (CN), 1736 (CO), 1629, 1582, 1431, 1402, 1271, 1168, 1086, 854. ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.85–2.00 (2.71H, m, β-CH_2 (*cis*), γ-CH_2 (*cis*, *trans*)); 2.02–2.08 (0.29H, m, β-CH_2 (*cis*)); 2.29–2.36 (1.00H, m, β-CH_2 (*cis, trans*)); 3.45 (0.87H, s, CH_3O (*cis*)); 3.47–3.51 (1.42H, m, δ-CH_2 (*trans*)); 3.62–3.67 (0.58H, m, δ-CH_2 (*cis*)); 3.68 (2.13H, s, CH_3O (*trans*)); 4.54–4.58 (1H, m, α-CH (*cis*, *trans*)); 7.98 (0.29H, d, *J* = 5.1, H-5 Py (*cis*)); 8.02 (0.71H, d, *J* = 5.1, H-5 Py (*trans*)); 8.88–8.90 (0.58H, m, H-2,6 Py (*cis*)); 8.92–8.93 (1.42H, m, H-6 Py (*trans*)). ^{13}C NMR spectrum (125 MHz, DMSO-d_6), δ, ppm: 23.4 (γ-C (*cis*)); 25.6 (γ-C (*trans*)); 29.9 (β-C (*trans*)); 31.8 (β-C (*cis*)); 47.7 (δ-C (*cis*)); 49.9 (δ-C (*trans*)); 52.9 (CH_3O (*trans*)); 53.3 (CH_3O (*cis*)); 59.8 (α-CH (*trans*)); 61.3 (α-CH (*cis*)); 116.0 (C-5 Py); 118.6 (CN (*trans*)); 119.2 (CN (*cis*)); 127.6 (C-3 Py (*cis*)); 127.8 (C-3 Py (*trans*)); 133.9 (C-4 Py (*trans*)); 134.2 (C-4 Py (*cis*)); 148.3 (C-2 Py (*cis*)); 148.8 (C-2 Py (*trans*)); 152.4 (C-6 Py (*cis*)); 152.6 (C-6 Py (*trans*)); 164.1 (O=C–N (*trans*)); 164.7 (O=C–N (*cis*)); 172.5 (COO (*trans*)); 172.7 (COO (*cis*)). Found, *m*/*z*: 260.1031 $[M+H]^+$. $C_{13}H_{14}N_3O_3$. Calculated, *m*/*z*: 260.1030.

Synthesis of methyl esters of *N*-{4-[amino(hydroxy-imino)methyl]pyridin-3-yl}carbonyl-substituted α-amino acids 6a–i (General method). To a stirred suspension of $NH_2OH·HCl$ (0.175 g, 2.5 mmol, 2.5 equiv) in MeOH (2.5 ml), $NaHCO_3$ (0.212 g, 2.5 mmol, 2.5 equiv) was added at room temperature. After 15 min, appropriate pyrrolo[3,4-*c*]pyridine **4a**–**h** or 4-cyanonicotinamide **3i** (1 mmol) was added. The obtained mixture was stirred until the TLC showed the absence of starting material. The silica (1 g) was added to the obtained solution, and the solvent then was evaporated under reduced pressure at ambient temperature. The residue was purified by flash column chromatography (gradient elution of MTBE–MeOH, 0:100–20:80) to afford the desired product as a solid.

Methyl [4-(*N*'-hydroxycarbamimidoyl)nicotinoyl]glycinate (6a). Yield 0.21 g (83%), pale-yellow foam. IR spectrum, ν, cm^{-1}: 3342, 2925, 1744, 1648, 1594, 1561, 1376, 1220, 937. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 3.68 (3H, s, CH_3O); 4.00 (2H, d, *J* = 5.8, CH_2); 5.82 (2H, s, NH_2); 7.54 (1H, d, *J* = 5.2, H-5 Py); 8.59 (1H, s, H-2 Py); 8.65 (1H, d, *J* = 5.1, H-6 Py); 8.90 (1H, t, *J* = 5.8, CONH); 9.90 (1H, s, OH). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm: 42.0 (CH_2); 52.9 (CH_3O); 123.7 (C-5 Py); 131.8 (C-3 Py); 140.5 (C-4 Py); 149.4 (C-2 Py); 150.5 (N=C–N); 151.5 (C-6 Py); 168.3 (O=C–N); 171.1 (COO). Found, *m*/*z*: 253.0933 $[M+H]^+$. $C_{10}H_{13}N_4O_4$. Calculated, *m*/*z*: 253.0931.

Methyl (*Z*)-[4-(*N*'-hydroxycarbamimidoyl)nicotinoyl]-L-alaninate (6b). Yield 0.21 g (80%), pale-yellow powder, mp 58–60°C. IR spectrum, ν, cm^{-1}: 3451, 3330, 2960, 1740, 1645, 1595, 1558, 1455, 1380, 1220, 940, 667. ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.36 (3H, d, *J* = 7.3, $CHC\underline{H}_3$); 3.67 (3H, s, CH_3O); 4.41–4.47 (1H, m, $C\underline{H}CH_3$); 5.82 (2H, s, NH_2); 7.53 (1H, d, *J* = 5.1, H-5 Py); 8.58 (1H, s, H-2 Py); 8.65 (1H, d, *J* = 5.1, H-6 Py); 8.83 (1H, d, *J* = 7.0, CONH); 9.88 (1H, s, OH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 17.8 ($CH\underline{C}H_3$); 49.0 ($\underline{C}HCH_3$); 53.0 (CH_3O); 123.7 (C-5 Py); 131.7 (C-3 Py); 140.5 (C-4 Py); 149.5 (C-2 Py); 150.5 (N=C–N); 151.4 (C-6 Py); 167.5 (O=C–N); 173.8 (COO). Found, *m*/*z*: 267.1092 $[M+H]^+$. $C_{11}H_{15}N_4O_4$. Calculated, *m*/*z*: 267.1088.

Methyl (*Z*)-[4-(*N*'-hydroxycarbamimidoyl)nicotinoyl]-L-valinate (6c). Yield 0.25 g (84%), colorless crystals, mp 171–173°C. IR spectrum, ν, cm^{-1}: 3508, 3377, 3282, 3040, 2976, 1740, 1638, 1597, 1572, 1365, 1259, 1201, 924, 662. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 0.94 (6H, d, *J* = 6.8, $2CH_3$); 2.07–2.15 (1H, m, β-CH); 3.67 (3H, s, CH_3O); 4.31–4.34 (1H, m, α-CH); 5.87 (2H, s, NH_2); 7.50 (1H, d, *J* = 5.1, H-5 Py); 8.58 (1H, s, H-2 Py); 8.65 (1H, d, *J* = 5.0, H-6 Py); 8.69 (1H, d, *J* = 8.0, CONH); 9.85 (1H, s, OH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 19.4 (CH_3); 20.0 (CH_3); 30.8 (β-CH); 52.8 (CH_3O); 59.1 (α-CH); 123.9 (C-5 Py); 131.6 (C-3 Py); 140.5 (C-4 Py);

149.8 (C-2 Py); 150.8 (N=C–N); 151.4 (C-6 Py); 167.7 (O=C–N); 172.7 (COO). Found, *m*/*z*: 295.1404 $[M+H]^+$. $C_{13}H_{19}N_4O_4$. Calculated, *m*/*z*: 295.1401.

Methyl (*Z*)-[4-(*N*'-hydroxycarbamimidoyl)nicotinoyl]-L-leucinate (6d). Yield 0.27 g (87%), pale-yellow foam. IR spectrum, ν, cm^{-1}: 3460, 3223, 2959, 2872, 1742, 1647, 1594, 1560, 1370, 1169, 939, 667. ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 0.90 (3H, d, *J* = 7.2, CH_3); 0.91 (3H, d, *J* = 7.2, CH_3); 1.54–1.73 (3H, m, CH_2, γ-CH); 3.69 (3H, s, OCH_3); 4.65–4.71 (1H, m, α-CH); 5.34 (2H, s, NH_2); 7.22 (1H, d, *J* = 5.1, H-5 Py); 7.86 (1H, d, *J* = 8.2, CONH); 8.45 (1H, d, *J* = 5.1, H-6 Py); 8.61 (1H, s, H-2 Py). ^{13}C NMR spectrum (100 MHz, $CDCl_3$), δ, ppm: 22.1 (CH_3); 23.1 (CH_3); 25.2 (γ-CH); 41.3 (CH); 51.8 (α-CH); 52.8 (CH_3O); 123.2 (C-5 Py); 130.7 (C-3 Py); 138.8 (C-4 Py); 149.2 (C-2 Py); 151.1 (N=C–N); 151.2 (C-6 Py); 167.2 (O=C–N); 173.7 (COO). ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 0.90 (3H, d, *J* = 6.5, CH_3); 0.92 (3H, d, *J* = 6.5, CH_3); 1.51–1.56 (1H, m) and 1.61–1.67 (1H, m, CH_2); 1.70–1.77 (1H, m, γ-CH); 3.67 (3H, s, OCH_3); 4.41–4.46 (1H, m, α-CH); 5.80 (2H, s, NH_2); 7.52 (1H, d, *J* = 5.1, H-5 Py); 8.57 (1H, s, H-2 Py); 8.65 (1H, d, *J* = 5.1, H-6 Py); 8.81 (1H, d, *J* = 7.6, CONH); 9.83 (1H, s, OH). ^{13}C NMR spectrum (125 MHz, DMSO-d_6), δ, ppm: 22.3 (CH_3); 23.8 (CH_3); 25.1 (γ-CH); 40.6 (CH); 51.8 (α-CH); 52.9 (CH_3O); 123.8 (C-5 Py); 131.6 (C-3 Py); 140.5 (C-4 Py); 149.6 (C-2 Py); 150.6 (N=C–N); 151.4 (C-6 Py); 167.6 (O=C–N); 173.7 (COO). Found, *m*/*z*: 309.1558 $[M+H]^+$. $C_{14}H_{21}N_4O_4$. Calculated, *m*/*z*: 309.1557.

Methyl (*Z*)-[4-(*N*'-hydroxycarbamimidoyl)nicotinoyl]-L-phenylalaninate (6e). Yield 0.32 g (93%), white powder, 176–178°C. IR spectrum, ν, cm^{-1}: 3390, 3308, 3065, 2955, 1748, 1731, 1663, 1594, 1518, 1372, 1214, 924, 704. ^{1}H NMR spectrum (600 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 3.00–3.13 (2H, m, CH_2); 3.64 (3H, s, OCH_3); 4.65–4.68 (1H, m, α-CH); 5.75 (2H, s, NH_2); 7.24 (1H, t, *J* = 7.1, H-4 Ph); 7.27–7.33 (4H, m, H Ph); 7.51 (1H, d, *J* = 5.1, H-5 Py); 8.38 (1H, s, H-2 Py); 8.63 (1H, d, *J* = 5.1, H-6 Py); 8.97 (1H, d, *J* = 7.7, CONH); 9.89 (1H, s, OH). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm: 37.7 (CH_2); 52.9 (CH_3O); 55.0 (α-CH); 123.7 (C-5 Py); 127.5 (C Ph); 129.3 (2C Ph); 130.1 (2C Ph); 131.4 (C-3 Py); 138.1 (C Ph); 140.3 (C-4 Py); 149.5 (C-2 Py); 150.6 (N=C–N); 151.4 (C-6 Py); 167.5 (O=C–N); 172.5 (COO). Found, *m*/*z*: 343.1397 $[M+H]^+$. $C_{17}H_{19}N_4O_4$. Calculated, *m*/*z*: 343.1401.

Methyl (*Z*)-[4-(*N*'-hydroxycarbamimidoyl)nicotinoyl]-L-tryptophanate (6f). Yield 0.35 g (91%), pale-orange powder, mp 176–178°C. IR spectrum, ν, cm^{-1}: 3332, 3044, 2952, 1754, 1620, 1597, 1562, 1347, 1215, 755, 669. ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 3.16–3.27 (2H, m, CH_2); 3.62 (3H, s, OCH_3); 4.68–4.72 (1H, m, α-CH); 5.81 (2H, s, NH_2); 7.01 (1H, dd, $^1J = {}^2J$ = 7.5, H-5 indole); 7.09 (1H, dd, ^1J = 7.8, ^2J = 7.5, H-6 indole); 7.26 (1H, d, *J* = 2.4, H-2 indole); 7.37 (1H, d, *J* = 8.1, H-7 indole); 7.52 (1H, d, *J* = 5.1, H-5 Py); 7.54 (1H, d, *J* = 7.9, H-4 indole); 8.47 (1H, s, H-2 Py); 8.64 (1H, d, *J* = 5.1, H-6 Py); 8.93 (1H, d, *J* = 7.5, CONH); 9.91 (1H, s, OH); 10.88 (1H, s, NH indole). ^{13}C NMR spectrum (150 MHz, DMSO-d_6), δ, ppm: 28.0 (CH_2); 52.9 (OCH_3); 54.6 (α-CH); 110.3 (C-3 indole); 112.4 (C-7 indole); 119.0 (C-4 indole); 119.4 (C-5 indole); 122.0 (C-6 indole); 123.8 (C-5 Py); 124.9 (C-2 indole); 128.1 (C-4a indole); 131.6 (C-3 Py); 137.1 (C-7a indole); 140.5 (C-4 Py); 149.6 (C-2 Py); 150.7 (N–C=N); 151.4 (C-6 Py); 167.6 (N–C=O); 173.0 (O–C=O). Found, *m*/*z*: 382.1507 $[M+H]^+$. $C_{19}H_{20}N_5O_4$. Calculated, *m*/*z*: 382.1510.

Methyl (*Z*)-[4-(*N*'-hydroxycarbamimidoyl)nicotinoyl]-L-aspartate (6g). Yield 0.27 g (82%), pale-yellow foam. IR spectrum, ν, cm^{-1}: 3453, 3350, 3042, 2956, 2852, 1738, 1649, 1594, 1557, 1439, 1373, 1222, 849, 667. ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 2.79–2.90 (2H, m, CH_2); 3.63 (3H, s, OCH_3); 3.67 (3H, s, OCH_3); 4.81–4.85 (1H, m, α-CH); 5.80 (2H, s, NH_2); 7.53 (1H, d, *J* = 5.1, H-5 Py); 8.53 (1H, s, H-2 Py); 8.65 (1H, d, *J* = 5.2, H-6 Py); 8.87 (1H, d, *J* = 8.1, CONH); 9.86 (1H, s, OH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 36.4 (CH_2); 49.8 (α-CH); 52.7 (CH_3O); 53.3 (CH_3O); 123.6 (C-5 Py); 131.6 (C-3 Py); 140.4 (C-4 Py); 149.3 (C-2 Py); 150.4 (N=C–N); 151.5 (C-6 Py); 167.5 (O=C–N); 171.5 (α-COO); 171.9 (β-COO). Found, *m*/*z*: 325.1143 $[M+H]^+$. $C_{13}H_{17}N_4O_6$. Calculated, *m*/*z*: 325.1143.

Methyl (*Z*)-[4-(*N*'-hydroxycarbamimidoyl)nicotinoyl]-L-glutamate (6h). Yield 0.29 g (86%), colorless foam. IR spectrum, ν, cm^{-1}: 3451, 3351, 3058, 2956, 1737, 1648, 1595, 1560, 1439, 1377, 1268, 1215, 938, 667. ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.89–1.96 (1H, m) and 2.03–2.10 (1H, m, β-CH_2); 2.46–2.49 (2H, m, γ-CH_2); 3.60 (3H, s, CH_3O); 3.67 (3H, s, CH_3O); 4.41–4.46 (1H, m, α-CH); 5.81 (2H, s, NH_2); 7.53 (1H, d, *J* = 5.1, H-5 Py); 8.57 (1H, s, H-2 Py); 8.66 (1H, d, *J* = 5.1, H-6 Py); 8.82 (1H, d, *J* = 7.5, CONH); 9.83 (1H, s, OH). ^{13}C NMR spectrum (125 MHz, DMSO-d_6), δ, ppm: 26.9 (β-CH_2); 36.4 (γ-CH_2); 52.3 (α-CH); 52.7 (CH_3O); 53.0 (CH_3O); 123.6 (C-5 Py); 131.7 (C-3 Py); 140.4 (C-4 Py); 149.5 (C-2 Py); 150.5 (N=C–N); 151.4 (C-6 Py); 167.8 (O=C–N); 172.8 (α-COO); 173.7 (γ-COO). Found, *m*/*z*: 339.1301 $[M+H]^+$. $C_{14}H_{19}N_4O_6$. Calculated, *m*/*z*: 339.1299.

Methyl [4-(*N*'-hydroxycarbamimidoyl)nicotinoyl]-L-prolinate (6i). Yield 0.24 g (81%), pale-yellow powder, mp 92–94°C. IR spectrum, ν, cm^{-1}: 3446, 3342, 2956, 2882, 1740, 1624, 1594, 1444, 1401, 1202, 1177, 943, 668. ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.81–1.95 (3.00H, m, β-CH_2 (*trans, cis*), γ-CH_2 (*cis*, *trans*)); 2.33–2.42 (1.00H, m, β-CH_2 (*trans, cis*)); 3.15–3.25 (1.30H, m, δ-CH_2 (*trans*)); 3.46 (1.05H, s, CH_3O (*cis*)); 3.50–3.60 (0.70H, m, δ-CH_2 (*cis*)); 3.69 (1.95H, s, CH_3O (*trans*)); 4.11–4.14 (0.35H, m, α-CH (*cis*)); 4.40–4.43 (0.65H, m, α-CH (*trans*)); 5.92 (1.30H, s, NH_2 (*trans*)); 5.98 (0.70H, s, NH_2 (*cis*)); 7.58 (0.35H, d, *J* = 5.2, H-5 Py (*cis*)); 7.61 (0.65H, d, *J* = 5.2, H-5 Py (*trans*)); 8.22 (0.35H, s, H-2 Py (*cis*)); 8.38 (0.65H, s, H-2 Py (*trans*)); 8.62 (0.35H, d, *J* = 5.2, H-6 Py (*cis*)); 8.66 (0.65H, d, *J* = 5.2, H-6 Py (*trans*)); 8.96 (1H, s, OH). ^{13}C NMR spectrum (125 MHz, DMSO-d_6), δ, ppm: 23.7 (γ-CH_2 (*cis*)); 25.4 (γ-CH_2 (*trans*)); 30.2 (β-CH_2 (*trans*)); 31.2 (β-CH_2 (*cis*)); 47.1 (δ-CH_2 (*cis*)); 49.2 (δ-CH_2 (*trans*)); 52.9 (CH_3O (*trans*, *cis*)); 59.3 (α-CH (*trans*)); 61.2 (α-CH (*cis*)); 122.3 (C-5 Py (*cis*)); 122.5 (C-5 Py (*trans*)); 131.3 (C-3 Py (*cis*)); 131.6 (C-3 Py (*trans*)); 138.3 (C-4 Py (*cis*)); 138.7 (C-4 Py (*trans*)); 148.1 (C-2 Py

(*trans*)); 148.3 (C-2 Py (*cis*)); 149.6 (N=C–N (*trans*)); 149.7 (N=C–N (*cis*)); 150.9 (C-6 Py (*cis*)); 151.0 (C-6 Py (*trans*)); 167.8 (N–C=O (*trans*)); 168.1 (N–C=O (*cis*)); 173.3 (COO (*trans*)); 173.5) (COO (*cis*)). Found, *m*/*z*: 293.1242 $[M+H]^+$. $C_{13}H_{17}N_4O_4$. Calculated, *m*/*z*: 293.1244.

X-ray structural study of compound 6c. The colorless crystals of compound **6c** ($C_{13}H_{18}N_4O_4$) are monoclinic. At 173K, *a* 9.5932(14), *b* 7.3711(10), *c* 9.9957(14) Å; β 95.633(10)°; *V* 703.41(17) Å^3; M_r 294.31; *Z* 2; space group $P2_1$; d_{calc} 1.390 g/cm^3; μ(MoKα) 0.105 mm^{-1}; *F*(000) 312. Intensities of 8285 reflections (2344 independent, R_{int} 0.061) have been collected on a Bruker APEX II diffractometer (graphite monochromated MoKα radiation, CCD detector, φ- and ω-scanning, $2\Theta_{max}$ 50°). The structure was solved by a direct method using the SHELXT package[16] and refined using SHELXL program.[17] Positions of the hydrogen atoms have been located from electron density difference maps and refined using riding model with $U_{iso} = nU_{eq}$ (n = 1.5 for methyl groups and n = 1.2 for other hydrogen atoms) of the carrier atom. Hydrogen atoms participated in the formation of N–H···O, N–H···N, and O–H···O hydrogen bonds have been refined using isotropic approximation. Full-matrix least-squares refinement against F^2 in anisotropic approximation for non-hydrogen atoms using 2344 reflections was converged to wR_2 0.165 (R_1 0.057 for 2156 reflections with $F > 4\sigma(F)$, *S* 1.071). The final atomic coordinates and crystallographic data for molecule **6c** have been deposited at the Cambridge Crystallographic Data Center (deposit CCDC 2280261).

Supplementary information file, containing ^{1}H and ^{13}C NMR spectra of the synthesized compounds is available at the journal's website http://link.springer.com/journal/10593.

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