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ORIGINAL PAPER

Methyl esters of 2-(*N*-hydroxycarbamimidoyl)benzoyl-substituted α-amino acids as promising building blocks in peptidomimetic synthesis: a comparative study

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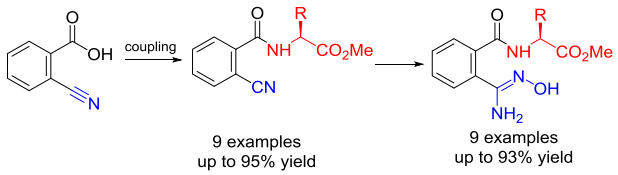
Abstract

An efficient and simple synthetic protocol for the synthesis of a number methyl esters of 2-(*N*-hydroxycarbamimidoyl)benzoyl-substituted (*S*)-α-amino acids via subsequent coupling and hydroxyamination of 2-cyanobenzamide derivatives has been developed. Comparative analysis of three pseudopeptide series based on 2-cyano- and 2-amidoxime-substituted benzoic acid and its pyridine and pyrazine counterparts has been provided and it has revealed a practical advantage of the benzoic acid derivatives due to their greater availability. The impact of the nitrogen atom in the aromatic ring on the *trans*/*cis*-amide equilibrium in the proline derivatives is discussed.

Graphical abstract



Keywords Amino acids · Carboxylic acids · Heterocycles · Isomers · Tautomerism · X-ray structure determination

Introduction

Amidoximes have gained an increased attention in recent decades because they have been shown to be reduced in vivo to the amidines [1–5] which are known to effectively replace guanidine group of arginine in the arginine mimetics for the treatment of blood-coagulation disorders [6]. Since that time, a new application of amidoximes not only as precursors for the synthesis of biologically active agents of amidine series, but also as prodrugs—precursors of amidine active forms that enhance oral bioavailability—has begun [7]. Amidoxime prodrugs such as platelet aggregation inhibitor sibrafiban [8] and a first oral direct thrombin inhibitor ximelagatran [9] have been developed (Fig. 1).

Amidoximes are also known to behave as nitric oxide donors (NO donors) because of their transformation by enzymes into amides with a subsequent release of NO [10]. L-Indospicine is a natural product amino acid analogue of L-arginine, and *N*-hydroxy-L-indospicine which is a carbon isostere of *N*-hydroxy-L-arginine acts as one of the best inhibitors of arginase [11–15] (Fig. 2).

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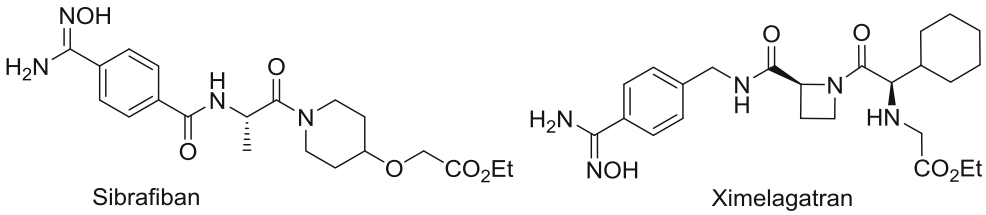


Fig. 1 Amidoxime prodrugs sibrafiban and ximelagatran

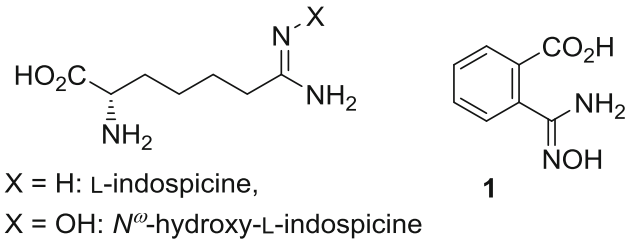


Fig. 2 L-Indospicine and its *N*-hydroxy derivative—carbon isosteres of L-arginine and *N*-hydroxy-L-arginine; 2-(*N*-hydroxycarbamimidoyl)benzoic acid **1**

Cyanobenzoic acids serve as precursors of molecules containing *N*′-hydroxycarbamimidoyl (amidoxime) motif. There are much data concerning synthesis and biological activity of 3- and 4-(*N*′-hydroxycarbamimidoyl)benzamide derivatives [16–22], whereas the derivatives of 2-(*N*′-hydroxycarbamimidoyl)benzoic acid (**1**) remain poorly studied. The acid **1** itself is unknown; thus, when 2-amidinobenzoic acid was re-aminated with hydroxylamine, heterocyclization resulted in 3-hydroxyimino-2,3-dihydro-1*H*-isoindol-1-one [23]. However, few derivatives of **1** were reported on their biological activity [24–26].

N-(2-Cyanobenzoyl)-substituted amino acids, namely the derivatives of glycine and DL-phenylalanine, were reported by Bergel and Stoke in 1957 [27] but no information on their preparation was given. Later, some *N*-2-cyanobenzoylated α-amino acid derivatives were prepared by peptide coupling techniques [28–36]. Their structural isomers 2-*N*-substituted 3-iminoisoindoline-1-ones are available by alkylation of 3-amino-1*H*-isoindole-1-one [37, 38] or its condensation with α-amino acid derivatives [39–41], from substituted phthalamic acid [42] or 2-iodobenzamides [43], and exhibited a wide range of biological activities [37, 39, 42, 44, 45]. Despite the known transformation of unsubstituted and *N*-substituted 2-cyanobenzamides into corresponding 3-iminoisoindolin-1-ones [46–51], the direct formation of compounds of this series from amino acids derived 2-cyanobenzamides remains unexplored.

Earlier, we have proposed a general approach to the synthesis of pyridin(pyrazin)yl-2(3) containing amidoxime pseudopeptides based on the coupling of 2(3)-cyanopyridine(pyrazine)carboxylic acid with methyl esters of L-α-amino acids followed by hydroxylamine treatment [52, 53]. At the same time, simpler molecules, based on a readily available 2-cyanobenzoic acid, were not studied. Therefore, the present work is a continuation of our research on the synthesis of the corresponding amino acid analogues with cyano and amidoxime groups in benzene ring and comparative studies on the synthesis and structure of these three series of potential arginine and indospicine mimetics.

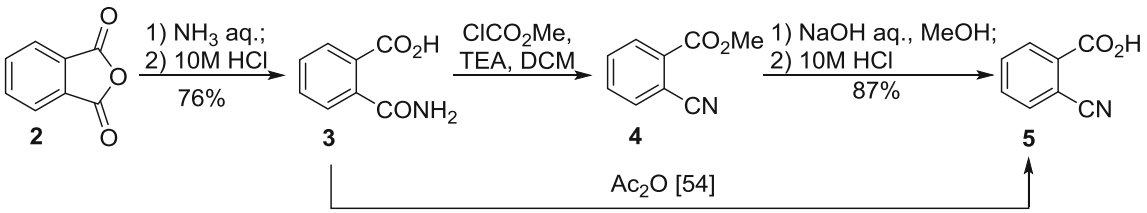
Results and discussion

For the synthesis of the target amidoxime-containing pseudopeptides, we employed the previously developed in our group a simple strategy based on the coupling of 2-cyanonicotinic or 2-cyanopyrazinic acids with methyl esters of L-α-amino acids followed by the conversion of the cyano group into amidoxime one [52, 53].

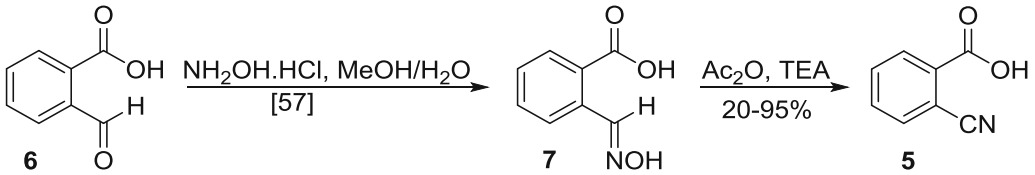
To prepare the starting acid **5**, different approaches known from the literature have been checked [54, 55]. The route which was previously successfully used for the preparation of pyridine- and pyrazine-containing cyano acids includes ammonolysis of phthalic anhydride (**2**) with the formation of phthalamic acid (**3**) [56], subsequent dehydration and simultaneous esterification with methyl chloroformate, and selective hydrolysis of methyl carboxylate **4** [55]. The approach via saponification of methyl 2-cyanobenzoate (**4**) was proved to be the best method which, with an improvement of acid preparation (up to 76% yield) and prolonged ester hydrolysis (up to 87%), provided the highest yields of the target acid **5** (Scheme 1). The conditions for each step were similar to that used for pyridine and pyrazine acids, although the highest total yield was achieved in the case of 2-cyanobenzoic acid (**5**). Compared with alternative method of dehydration of phthalamic acid (**3**) with Ac_2O [54], this method allows obtaining a pure final product **5** without phthalimide impurities.

Another method we attempted to develop was dehydration of oxime of 2-formylbenzoic acid (**6**) [57] to cyano acid **5** with acetic anhydride (Scheme 2). It was carried out under conditions similar to those used in the preparation of phthalamic acid (**3**) [54]. Since the yields were not high enough (20–30%) and despite once it was possible to reach 95% yield of acid **5**, this approach was found to be ineffective.

Scheme 1



Scheme 2



It should be noted that with prolonged storage at ambient temperature, the acid **5** undergoes slow hydrolysis with a phthalimide formation. The purity control of the compound **5** is best assessed by the presence/absence of phthalimide 4-7-H four-proton singlet at the 7.8 ppm region in the ^{1}H NMR spectrum in DMSO-d_6 solution.

The acid **5** was coupled with a series of methyl esters of L-α-amino acids with activating agent 1-ethyl-3-(dimethylaminopropyl)carbodiimide hydrochloride (EDCI) in the presence of hydroxybenzotriazole (HOBT) and TEA in DCM medium according to a previously developed protocol [52]. A characteristic feature of this reaction is the formation in most cases of a mixture of coupling products: methyl 2-[[(2-cyanophenyl)carbonyl]amino]alkanoates **8** and their tautomers endo-*N*- substituted methyl 2-(1-imino-3-oxo-1,3-dihydro-2*H*-isoindol-2-yl)alkanoates **9** derived from subsequent ring closure (Scheme 3, Table 1). This result differs from the described amination of the methyl esters of 2-cyanobenzoic or 2-cyanopyridine carboxylic acids with amines or alkylhydrazines, which was accompanied by the formation of only cyclic products with an amino residue at the exocyclic atom of nitrogen [38]. Total yields of esters **8/9** were significantly higher (90–95%) than for pyridine (58–85% [52]) and pyrazine derivatives (51–68% [53]).

The formation of the cyclic isomeric iminoisoindolones **9a**–**9h** as minor products together with the desired 2-cyanobenzamides **8a**–**8h** was detected by TLC of the reaction mixture. The individual isomers were isolated by column chromatography (gradient elution of ethyl acetate/petroleum ether 0:100–60:40). Chromatographic separation of crude Trp **8g/9g** and Glu **8h/9h** derivative mixtures made it possible to obtain only **8 g** and **8 h** compounds in an individual form.

The degree of tautomeric conversion of 2-cyanobenzamides **8a**–**8h** into **9a**–**9h** depends on the reaction time and the branching of the amino acid side chain. The Gly derivative **8a** has been completely transformed into iminoisoindolone **9a** in less than 1 day, and for the Leu derivative **8d** conversion to the corresponding cyclic isomer **9d** has been completed upon stirring the reaction mixture at rt for 2 days.

An attempted coupling of 2-cyanobenzoic acid (**5**) with methyl ester of alanine under microwave irradiation at

Scheme 3

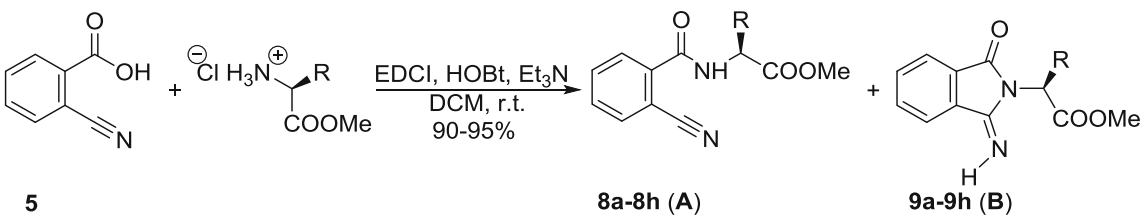


Table 1 Coupling of 2-cyanobenzoic acid (**5**) with methyl esters of (*S*)-α-amino acids and the comparison with coupling of 2-cyanopyridine and 2-cyanopyrazine series

Entry	AA	Products	Yield[a]/% **8A**:**9B**	Py [52] **A**:**B**	Pz [53] **A**:**B**
1	Gly	**8a**:**9a**	74:15	–[d]:58	–
2	Ala	**8b**:**9b**	80:9	15:58	23:28
3	Val	**8c**:**9c**	85:6	37:34	–
4	Leu	**8d**:**9d**	91:4	9:76	–
5	Met	**8e**:**9e**	83:7	–	–
6	Phe	**8f**:**9f**	86:3	–[d]:76	3:65
7	Trp	**8g**:**9g**	92:0[b]	–[d]:83	–
8	Glu	**8h**:**9h**	84:0[c]	–	–
9	Pro	**8i**	95	74	49

[a]Yield after column chromatography separation

[b]Only one compound **8g** was isolated, **9g** was detected by TLC and ^{1}H NMR in the crude product

[c]Only one compound **8h** was isolated, **9h** was detected by TLC and ^{1}H NMR in the crude product

[d]The open form **A** was not isolated

50 °C shortened the processing time to 5–10 min. However, in this case, in spite of the rapid conversion of acid **5** into the final mixture consisting of amide **8b** and iminoisoindolone **9b** in approximately a 1:1 ratio, formation of phthalimide as a by-product was more intensive according to TLC.

The electronic nature of the aromatic ring significantly affects the tautomeric ratio of two forms **8A/9B**. If in the case of cyanobenzamides the open form **8A** much more predominates, then for the compounds of the series of π-deficient pyridine, the cyclic form of iminoisoindolone **B** was much more prevalent, and only the cyclic ester was isolated for the Gly, Phe, and Trp derivatives [52]. A similar situation was observed in the pyrazine series: for Ala, the cyclic form **B** content was slightly larger (23:28), whereas in the case of Phe it predominated almost completely (3:65) [53]. Evidently, the presence of an electronnegative nitrogen atom in the β-position to the cyano group leads to its activation to the nucleophilic attack by the nitrogen atom of the amide fragment to afford pyrrolidine ring closure.

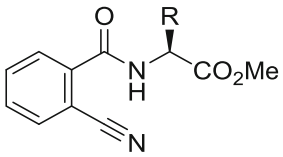
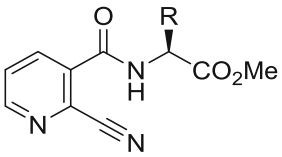
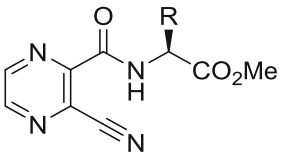
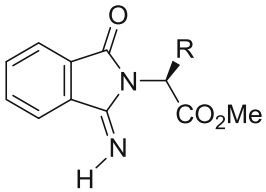
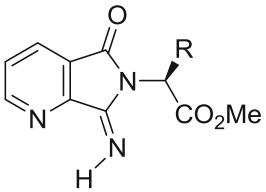
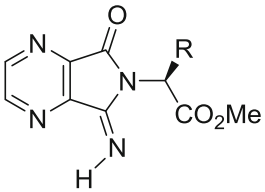
The assignment of isomers, as in the case of aza analogues, has been made based on the chemical shifts of the NH protons signals in their ^{1}H NMR spectra in $CDCl_3$ (Table 2). In the spectra of iminoisoindolones **9a**–**9f**, there are broadened single-proton NH signals of reduced intensity at δ = 8.40–8.90 ppm, while the signals of amide CONH group protons of **8a**–**8f** appear in the form of doublets or broadened singlets at 6.60–7.20 ppm. For pyridine derivatives, the open form reveals NH signals in the region of 7.01–7.15 ppm, whereas for pyrazine amides, at 8.20–8.26 ppm. Similarly, cyclic aza isomers exhibit signals of NH protons in the region of 9.29–9.37 ppm (pyridine) and 9.46–9.51 ppm (pyrazine) that are shifted downfield compared to benzene species **9a**–**9f** (8.56–8.65 ppm). The conclusion that can be drawn is that replacing the phenylene ring by a more electron-deficient pyridine and an even greater degree of pyrazine nucleus results in a significant shift of the NH signals to a low field.

One of the reasons that causes such shifts can be the *E* configuration of the substituents at C=N bond and *syn* orientation of the NH atom to the aromatic ring of the iminoisoindolones **9**. The NH proton that is oriented towards the aromatic ring and located in the same plane is influenced by the anisotropic effect of the aromatic ring current so that its signal is found at much lower field (8.56–8.65 ppm). The same deshielding effect additionally can be caused by nitrogen atoms of the pyridine and pyrazine rings of corresponding analogues. Significant differences in the shifts of the carbon atoms signals which belong to the open **A** and cyclic form **B** were observed in the ^{13}C NMR spectra. The signal of the CN group of the amides of **8** was manifested at about δ = 118 ppm, and in the spectra of iminoisoindolones **9** a signal of about 160 ppm appeared instead of it, which can correspond to the carbon atom of the N–C=N chain. Carbon which was bound to the CN group and resonated in a region of 110–112 ppm shifted to a lower field of 130–132 ppm.

The bond configuration is determined by X-ray diffraction of the cyclic Gly **9a** and Ala **9b** derivatives (Fig. 3). It is the same as C=N bond *E* configuration of the pyridine series [52].

The **9a** compound was previously studied by single-crystal X-ray diffraction analysis at 200 K [43]; cell parameters are close to the current experimental data at 298 K. The distribution of electron density in N(2)=C(7)–N(1)–C(8)=O(1) fragment is rather similar in **9a** and **9b**, revealing minor π-conjugation. However, the N(2)=C(7) and N(1)–C(8) bonds in **9a** (1.263(5) and 1.388(5) Å) are little longer than corresponding bonds in **9b** (1.255(2) and 1.372(2) Å). The H atom at N(2) has *syn* orientation with respect to the benzene ring in both compounds. Both N(1)

Table 2 Comparison of δ (NH) chemical shift values ($CDCl_3$) in the open **A** and cyclic **B** forms

AA, R	Open form **A**, δ (NH)/ppm ($CDCl_3$)		
			
Ala, R=Me	6.88	7.15	8.26
Phe, R=Bn	6.64	–[a]	8.20
Cyclic form **B**, δ (NH)/ppm ($CDCl_3$)			
			
Ala, R=Me	8.56	9.34	9.51
Phe, R=Bn	8.65[b]	9.29	9.46

[a]Was not isolated

[b]Was observed in a crude ^{1}H NMR spectrum but was not isolated

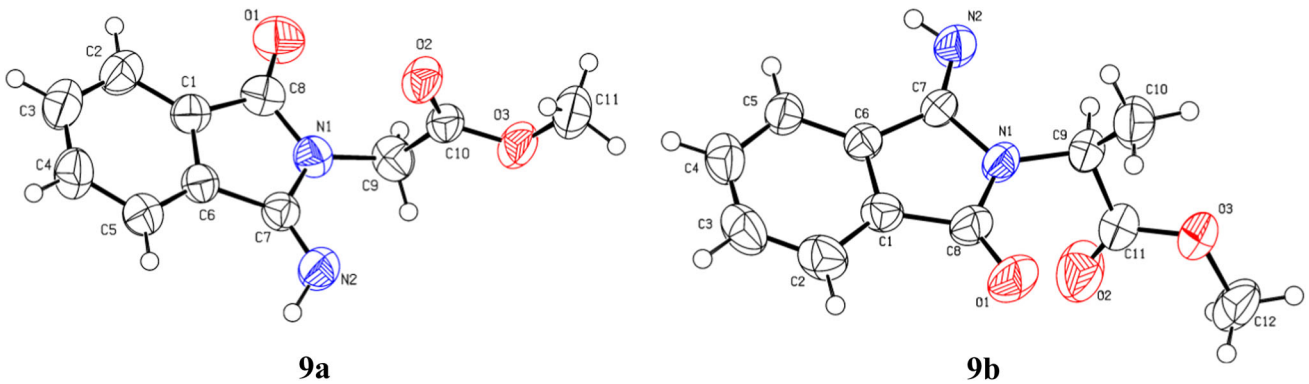


Fig. 3 Molecular structure of **9a** and **9b** according to X-ray diffraction study with the atom numbering used in the crystallographic analysis. Atoms are shown as 50% thermal ellipsoids

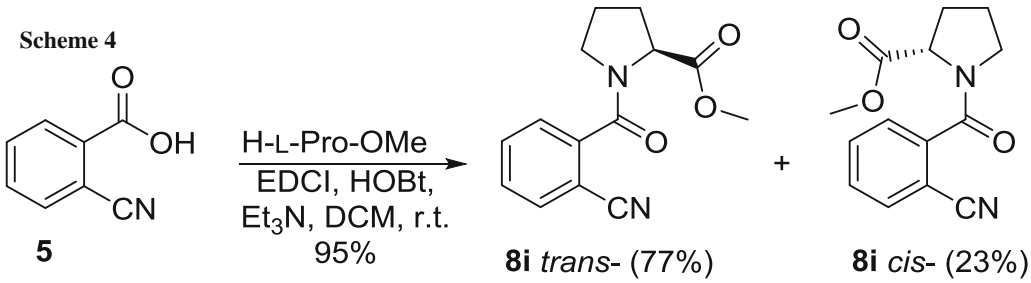
atoms are planar (sum of the valence angles centred on N(1) is 359.3(9)° and 360.0(6)° in **9a** and **9b**, respectively). The carboxylic substituent at N(1) reveals –sc orientation in **9a** but –ac orientation in **9b** with respect to the C(7)–N(1) endocyclic bond (the C(7)–N(1)–C(9)–C(10) torsion angle is − 80.9° in **9a** while the corresponding C(7)–N(1)–C(9)–C(11) torsion angle is −125.7° in **9b**). In the crystal, both compounds form chains along the [101] crystallographic direction in **9a** and [100] in **9b**, due to the N–H…O′ intermolecular hydrogen bonds (N(2)–H(2)…O(2)′ (x + 0.5, 1 − y, z + 0.5, H…O′ 2.32 Å, N–H…O′ 157°) in **9a**; N(2)–H(2)…O(1)′ (x − 1, y, z, H…O′ 2.33 Å, N–H…O′ 178°) in **9b**.

It should be noted that iminoisoindolones **9a**–**9g** were obtained recently by another more complicated synthetic approach after the start of our research. They were isolated as a sole product from the reaction of 2-iodobenzamides derived from amino acid methyl/ethyl esters and benzyl cyanide in the presence of potassium phosphate and copper(I) iodide catalyst [43]. More stringent reaction conditions (heating in DMSO at 100 °C for 11–25 h) probably contributed to the formation of only one tautomer of iminoisoindolone **9**.

L-Proline derivative **8i** for which the ring closure is not possible was isolated in 95% yield which significantly exceeds the yields of ProOMe transformations with cyanopyridine (74%, DCM, rt, [52]) and cyanopyrazine acids (49%, THF, 0°C, [53]) (Scheme 4).

Its ^{1}H and ^{13}C NMR spectra exhibit the double set of signals of all atoms due to *trans*,*cis*-amide isomerism.

Scheme 4



According to ^{1}H, ^{13}C NMR, HSQC, NOESY spectra of **8i**, the approximate ratio of the rotamers is estimated. Cross-peaks corresponding to the spatial interaction between the proton at C6 position of the benzene ring and the proton at C5 position of the pyrrolidine unit indicate that the major product is the *trans*-isomer ($\approx$ 77%), and the minor is *cis*-isomer ($\approx$ 23%), accordingly. This isomeric *trans,cis* ratio is similar to that of 77:23 ratio of the pyridine analogue [52], whereas pyrazine counterpart exhibits in solution the *cis*-amide conformation in much higher content when both forms are nearly equally populated: *trans*/*cis*—54:46 due to additional 4-N atom that involved in noncovalent intramolecular $n \rightarrow \pi^*$ interaction with C_{co} of the pyrrolidine ring [53].

The N(2) atom of pyrrolidine ring in **8i** is planar [sum of the valence angles centred on N(2) is 359.4(6)°] due to the conjugation in amide fragment N(2)–C(8)=O(1) (Fig. 4). The pyrrolidine ring adopts not envelope but twist conformation. Atoms C(10) and C(11) are disordered over two mirror symmetrical twist conformations (deviations of the atoms are + 0.32 Å and − 0.29 Å for C(10A) and C(11A), − 0.34 and + 0.15 for C(10B) and C(11B), respectively). The carboxymethyl substituent at C(12) atom is located in

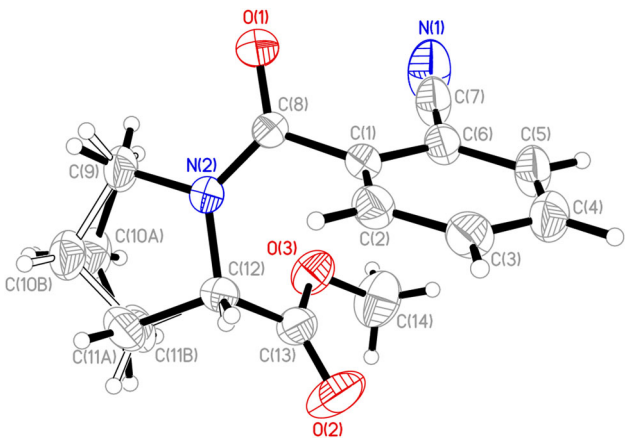
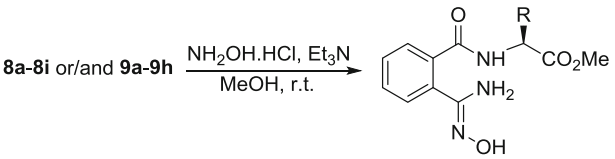


Fig. 4 Molecular structure of *cis*-**8i** according to X-ray diffraction study with the atom numbering used in the crystallographic analysis. Atoms are shown as 40% thermal ellipsoids

Table 3 Synthesis of amidoxime derivatives **10a–10i**



Entry	AA	R	Product	Yield[a]/%
1	Gly	H	**10a**	72
2	Ala	Me	**10b**	75
3	Val	*i*-Pr	**10c**	83
4	Leu	*i*-Bu	**10d**	89
5	Met	$(CH_2)_2SCH_3$	**10e**	82
6	Phe	Bn	**10f**	88
7	Trp	CH_2-3-indolyl	**10g**	93
8	Glu	$(CH_2)_2CO_2H$	**10h**	81
9	Pro		**10i**	92

[a]Yield after column chromatography purification

axial orientation (the C(9)–N(2)–C(12)–C(13) torsion angle is 116.2(2)°) and its carboxylic fragment is turned to the N(2)–C(12) endocyclic bond [the N(2)–C(12)–C(13)–O(2) torsion angle is 153.3(2)°]. The aryl ring of the substituent at the N(2) atom is located in *cis*-conformation to the N(2)–C(12) endocyclic bond and is rotated with respect to the plane of the amide fragment (the C(12)–N(2)–C(8)–C(1) and N(2)–C(8)–C(1)–C(2) torsion angles are − 9.0(3)° and − 62.5(2)°, respectively).

In the crystal phase, molecules of **8i** are organized in 3D structure due to non-specific and weak C–H…π interactions (H(10D)…C(5)′ − x, 0.5 + y, − 1.5 − z. H…C' 2.73 Å, sum of the van der Waals radii is 2.87 Å [58]).

Further, amidoximes **10a–10i** were obtained in good yields from corresponding methyl esters **8a–8i** and **9a–9h** (Table 3). Similar to aza-analogues [52, 53], the pyrrolidine cycle of iminoisoindolones **9a–9f**, taken in an individual form under the reaction conditions is opened with the formation of the desired amidoximes **10a–10h**. Therefore, upon treatment with hydroxylamine of the crude amides **8a–8h**, which contained an impurity of the

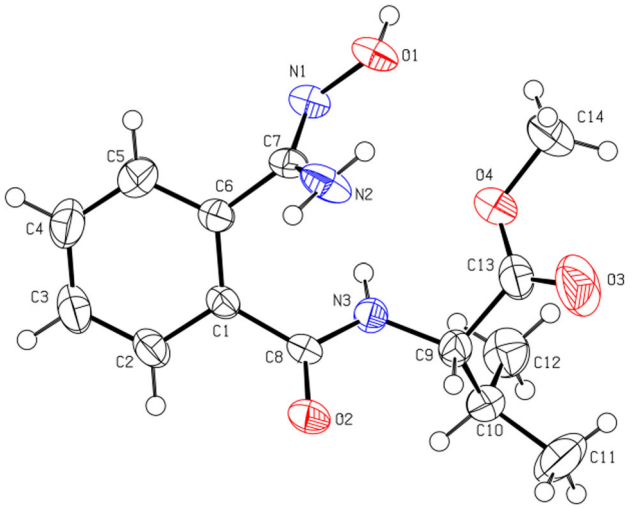


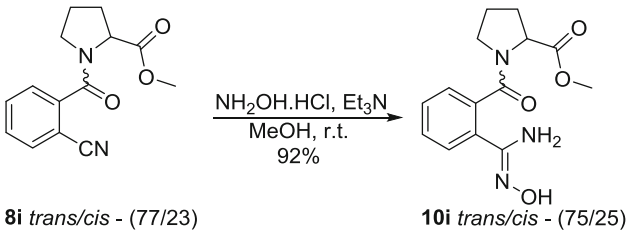
Fig. 5 Molecular structure of **10c** according to X-ray diffraction study with the atom numbering used in the crystallographic analysis. Atoms are shown as 50% thermal ellipsoids

corresponding iminoisoindolones **9a**–**9h**, their complete conversion to the final amidoximes **10a**–**10h** was observed. The treatment with hydroxylamine hydrochloride and Et_3N was carried out in MeOH at 0 °C followed by stirring at ambient temperature for 1.5–8 h. The crude products were purified by silica gel flash column chromatography to afford amidoximes **10a**–**10i** in 72–93% yield which exceeded the yields for pyridine (67–79% [52]) and pyrazine analogues (63–84% [53]).

This conclusion was proved by X-ray diffraction data of amidoxime **10c** (Fig. 5). Compounds **10a**–**10i** in their ^{1}H NMR spectra in DMSO-d_6 exhibit characteristic chemical shifts: sharp two-proton singlet of amidoxime NH_2 group at δ = 5–6 ppm, OH signal at 9–10 ppm, and signal of amide CONH of **10a**–**10 h** at 8–9 ppm. In addition, in the ^{13}C NMR spectra, the signals due to CN group disappeared, instead, a new signals at δ = 151–154 ppm that originated from carbon atoms of N–C=NOH group were observed. The IR spectra of amidoximes **10a**–**10i** exhibited broad absorption bands at 3000–3500 cm^{-1}, due to NH, NH_2, and OH groups, sharp bands at 1720–1750 (C=O) and 1620–1640 (C–N) cm^{-1} and the lack of characteristic cyano group band at 2223–2230 cm^{-1}.

The vicinal amide and *N*-hydroxycarbamimidoyl groups are rotated with respect to the plane of benzene ring (angles between planes are 47.3° and 61.8°, respectively; Fig. 5). Both N(2) and N(3) atoms adopt planar configuration (sum of the valence angles centred on these atoms are 355(7)° and 360.0(3)°, respectively). The bond lengths in *N*-hydroxycarbamimidoyl fragment differ negligibly from the corresponding mean values (N(1)–O(1) 1.430(2) Å and N(2)–C(7) 1.350(2) are elongated against the mean values of 1.415 Å and 1.340 Å, respectively [59]). The anti

Scheme 5



8i *trans/cis* - (77/23) **10i** *trans/cis* - (75/25)

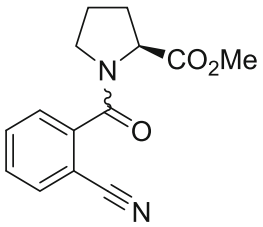
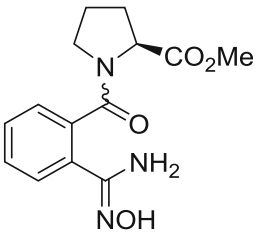
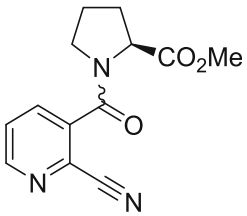
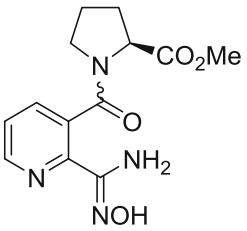
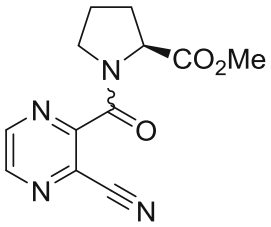
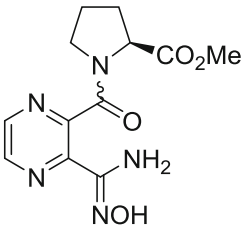
orientation of the H(1) atom at O(1) with respect to NH_2 group is due to the intramolecular attractive contact H(2A)…O(1) 2.23 Å (sum of van der Waals radii [58] is 2.46 Å). The ester fragment of the substituent at the carbamide group is located in *ac*-conformation to the C(8)–N(3) bond and its carboxylic group is turned to the N(3)–C(9) bond (the C(8)–N(3)–C(9)–C(13) and N(3)–C(9)–C(13)–O(4) torsion angles are 131.8(2)° and 32.6(2)°, respectively). The isopropyl group is turned in such a way that the N(3)–C(9)–C(10)–H10 torsion angle is 45° (Table 1, SI).

Like the starting Pro CN-derivative **8i**, amidoxime **10i** is a mixture of *cis*- and *trans*-amide conformers, since two sets of signals with approximately the same *trans*/*cis* ratio of 75:25 belonging to individual isomers were observed in the ^{1}H and ^{13}C NMR spectra (Scheme 5). As in the cases of pyrazine Pro derivative [53], the general tendency to adopt *trans*-conformation is observed (Table 4).

As a by-product in these conversions, 1*H*-isoindole-1,3(2*H*)-dione oxime (**11**) was isolated in each case (Scheme 6). According to TLC, it was formed in an insignificant amount already in the reaction mixture. However, its content increased during the extraction of amidoximes from the reaction mixture with EtOAc and their further chromatographic purification, which led to decreasing the yields of compounds **10a**–**10i**. The higher the amount of oxime **11** monitored by TLC, the less branched was the amino acid side chain: Gly > Ala > Pro > Met > Val > Leu > Phe > Trp. The gradual formation of oxime precipitate **11** occurred even with prolonged exposure to solutions of purified **10a**–**10i** in EtOAc at room temperature, and when they were heated. In the solid state, compounds **10a**–**10i**, in particular those containing the less branched amino acid side chains—Gly, Ala and Pro—partially decomposed, and much more when the substances were contaminated with residual solvent. Transformation of glycine derivative **10a** into oxime **11** was completed in 56 h upon stirring its methanolic solution at rt (TLC).

Oxime **11** was first obtained by the reaction of hydroxylamine with ethyl 2-cyanobenzoate [60], and later

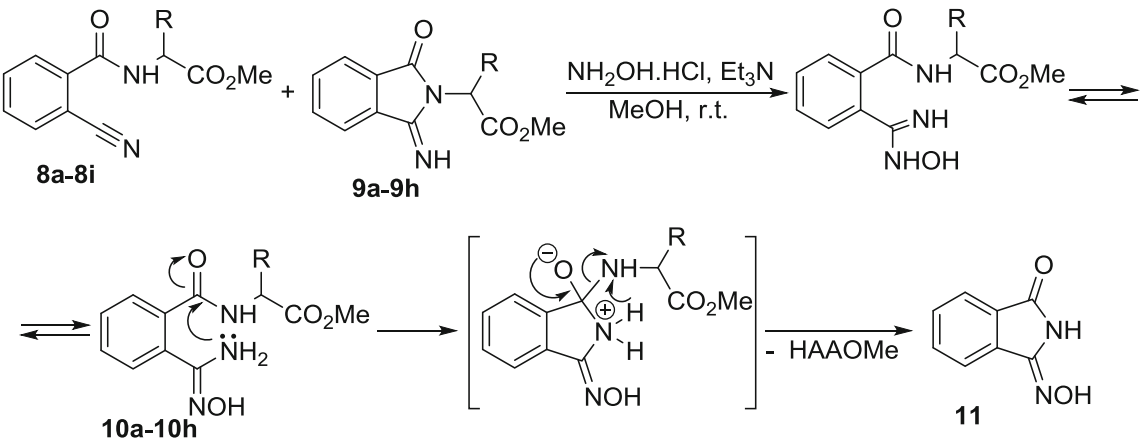
Table 4 *Trans/cis*-distribution in Pro pseudopeptides **8i**, **10i** with those obtained from 2-cyanonicotinic and 2-cyanopyrazinic acids

Cyano derivative	Yield[a]/%	*Trans/cis* ratio	Oxime derivative	Yield[a]/%	*Trans/cis* ratio
	95	77/23		92	75/25
	87	77/23		70	68/32
	49	54/46		93	74/26

Taken from [53]

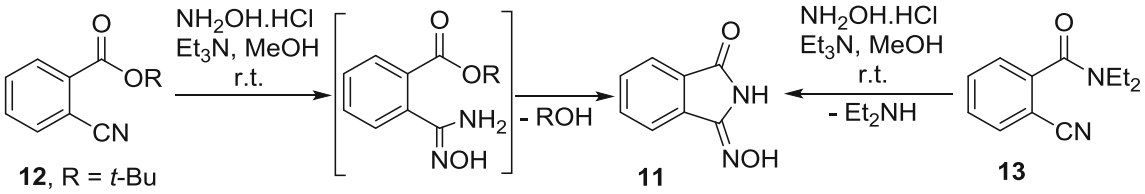
[a]Isolated yield

Scheme 6

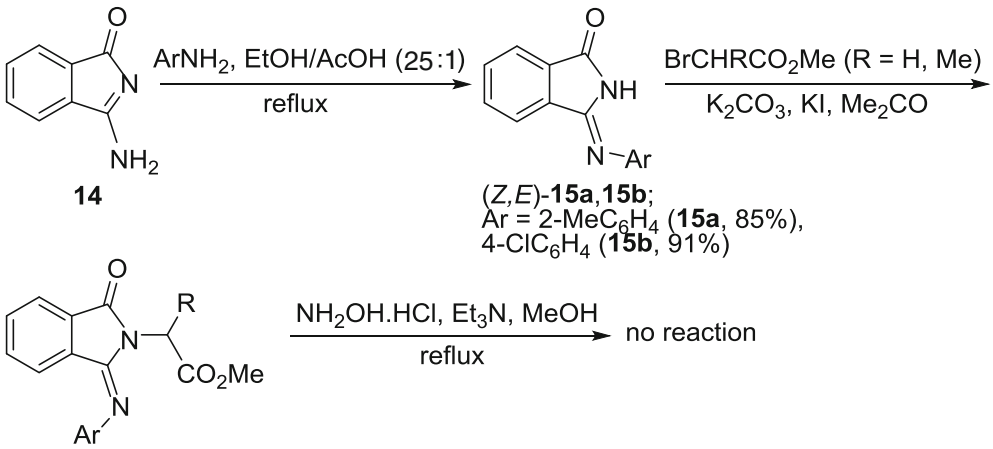


from unsubstituted 3-aminoisoindol-1-one by heating with hydroxylamine hydrochloride in methanol [47]. Therefore, we investigated the reaction of bulk *tert*-butyl 2-cyanobenzoate **12** with hydroxylamine (Scheme 7). The pyrrolidine ring closure leading to oxime **11** also occurred although in this case the reaction was completed within more than 1 day at room temperature. It should be noted that during the initial stage of the reaction, the intermediate esters that have not been isolated were probably seen as additional spots on TLC. An attempt to convert diethylamide of 2-cyanobenzoic acid **13** into the corresponding amidoxime also failed, leading exclusively to compound **11** in 2 days.

Scheme 7



Scheme 8



Taking these facts into account, it can be assumed that the ease of formation of the cyclic amidoxime **11** from amides **10** can be determined not only by steric hindrance, but electronic effects as well, in particular by the presence of an *N*-(methyl alkanoate) group that prevents cyclization.

To investigate the possibility of the pyrrolidine ring opening of 3-*N*-aryl-substituted analogues of isoindolones **9**, *N*-aryl imines **16a**–**16d** were synthesized by (*Z,E*)-3-arylimino-2,3-dihydro-1*H*-isoindol-1-ones **15a**, **15b** [61, 62] alkylation according to the known protocol [38] (Scheme 8). Like unsubstituted at the exocyclic nitrogen isoindolones **9a**–**9 h**, their *N*-arylated analogues **16a**–**16d** exist as *E*-isomers of the substituents at C=N bond [61]. This conclusion was reached based on the observation that the 4-H proton signals are considerably shifted upfield because of the shielding effect of *N*-aromatic ring [61, 63–65]. However, attempts to transform them into the corresponding *N*-arylamidoximes upon treatment with hydroxylamine under the above-described conditions were unsuccessful. Furthermore, the heating of the reaction mixture at 50 °C for several hours did not afford the desired product. In each case, only the starting compounds were quantitatively isolated. Probably, the steric hindrance created by the bulk aryl residue prevents the 3-C atom of the isoindole nucleus of compounds **16a**–**16d** from attack by hydroxylamine.

Conclusion

Comparative analysis of pseudopeptides based on 2-cyano- and 2-amidoxime-substituted benzoic acid and its pyridine and pyrazine counterparts revealed that the developed approach allows to obtain the desired compounds in high yields and benefits from operational simplicity and availability of starting materials. These compounds can be used as pseudopeptide building blocks, in particular, amidoximes as precursors of amidines in L-arginine and L-indospicine mimetics.

In the series of cyano-pseudopeptides, 2-cyano group of benzoic acid under employed conditions exhibits lower reactivity towards pyrrolidine ring closure reaction with amide unit in contrast to its aza analogues. NMR studies of a series of proline-containing peptides reveal a pronounced

effect of the aromatic ring nature upon the *cis*-to-*trans* ratio of the adjacent amide bond in solution. 2-Cyano-substituted benzoic and nicotinic acid amides exhibit a preference of the proline residue for the *trans* form, whereas 2-cyanopyrazine counterpart adopts the *cis*-amide conformation in much higher content of 46% when both forms are nearly equally populated in solution. Thus, the hypothesis that the presence of the extra nitrogen atom of pyrazine ring favours the *cis*-conformation for cyano derivatives was confirmed. The conversion of cyano group into amidoxime unit revealed almost the same isomeric ratio with a preference of the proline residue for the *trans* form of the entire three types of amidoxime pseudopeptides.

Experimental

Melting points were determined on a Boetius microscope hot plate apparatus. Elemental analyses (C, H, N, S) were conducted using the Vario Micro Cube; their results were found to be in good agreement (± 0.3%) with the calculated values.

LC/MS spectra were recorded using a system that consisted of a high-performance liquid chromatograph (Agilent 1100 Series) equipped with a diode-matrix and mass-selective detector (Agilent LC/MSD SL); ionization method: chemical ionization under atmospheric pressure (APCI). IR spectra were recorded with a PerkinElmer Spectrum BX FTIR Spectrometer with KBr pellets. ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance 300 model spectrometer operating at 300 and 75 MHz, and Bruker Avance 400 model spectrometer at 400 and 100 MHz, respectively, in $CDCl_3$ and DMSO-d_6 at 20 °C with tetramethylsilane as an internal reference. ^{1}H and ^{13}C chemical shifts (δ) are given in parts per million (ppm) relative to the residual solvent peak. HMBS spectra were recorded on a Bruker Avance 300 model spectrometer at 300 and 75 MHz. HSQC and NOESY spectra were recorded on a Bruker Avance 500 spectrometer at 500 and 125 MHz. Coupling constants J were directly taken from the spectra and are not averaged. TLC analyses were carried out on silica gel-coated aluminium sheets (Merck) and spots were visualized with UV light. Preparative column chromatography was carried out using silica gel 60, 40–63 μm.

Crystal structure determination

Diffraction data for crystals of **8i**, **9a**, **9b**, and **10c** were collected on an Xcalibur-3 diffractometer (MoKα radiation, λ = 0.71073 Å, CCD-detector, graphite monochromator, ω-scanning) at room temperature. Empirical absorption correction was provided with a multi-scan method using spherical harmonics, implemented in the SCALE3 ABSPACK scaling algorithm of the CrysAlisPro program package [66]. Structures were solved by direct methods and refined against F^2 within anisotropic approximation for all non-hydrogen atoms using the OLEX2 [67] program package with SHELXT and SHELXL modules [68]. **9a** structure was refined as a twin with twinning matrix (0.124, 0, − 0.876, 0, − 1, 0, −1.124, 0, − 0.124) using HKLF5 instruction [refined BASF parameter is 0.362(8)]. In **8i**, the C10 and C11 atoms of pyrrolidine ring were disordered over two symmetrical twist conformations with relative component ratio of 0.59:0.41. The C(10A)–C(11A) and C(10B)–C(11B) bonds were restrained to have fixed length of 1.52(1) Å, and all neighbouring bonds C(9)–C(10A,B) and C(12)–C(11A,B) were restrained to be approximately of the same length to within 0.01 Å. All H atoms were placed in idealized positions and constrained to ride on their parent atoms with U_{iso} = 1.2U_{eq} (1.5 U_{eq} for OH and CH_3 groups) except the H atoms at N(2) atoms in **9b** and **10c** that were refined isotropically. Details of the data collection and processing, structure solving and refinement are summarized in Table 2 (SI). CCDCs 1836420–1836423 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

2-Carbamoylbenzoic acid (3) [56] A suspension of 10.0 g phthalic anhydride (**2**, 67.5 mmol) in 50 cm^3 28% NH_4OH was stirred at 50 °C until the reagent was dissolved. After cooling to 0 °C, the precipitated product was separated off. To the filtrate 50 cm^3 acetone was added and the additional amount of precipitate was obtained. The combined precipitates were dissolved in 50 cm^3 water and re-precipitation was performed with addition of 10 M HCl (dropwise to pH 3) while cooling. The precipitate was filtered off, washed with acetone and dried to afford 8.49 g (76%) acid **3** as a colourless crystalline powder. M.p.: 144–145 °C ([49] 145 °C);

Methyl 2-cyanobenzoate (4) [55] To a stirred suspension of 3.52 g compound **3** (21.3 mmol) in 50 cm^3 DCM at 0 °C 6.23 cm^3 TEA (44.7 mmol) and 3.62 cm^3 methyl chloroformate (46.8 mmol) were added. After stirring at room temperature overnight, the reaction mixture was concentrated under reduce pressure, diluted with 100 cm^3 DCM, washed with 3 × 100 cm^3 H_2O and 100 cm^3 brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was washed with 100 cm^3 petroleum ether and dried to afford 3.38 g (98%) compound **4** as a colourless crystal. M.p.: 47–49 °C (Ref. [49] 47 °C); ^{1}H and ^{13}C NMR spectra were found to be identical with the ones described in [49].

2-Cyanobenzoic acid (5) [55] The mixture of 3.29 g methyl 2-cyanobenzoate **4** (20.4 mmol), 60 cm^3 MeOH, and 22 cm^3 1 M NaOH was stirred overnight, and then concentrated in vacuo. The residue was diluted with 100 cm^3 H_2O and 100 cm^3 DCM, and then acidified with 22 cm^3 1 M HCl under cooling. The resulting precipitate was collected, washed with 100 cm^3 H_2O, 100 cm^3 petroleum ether and dried to afford 2.60 g (87%) 2-cyanobenzoic acid **5** as a white crystalline powder. M.p.: 228–230 °C ([54] 227–228 °C). ^1H and ^{13}C NMR spectra were found to be identical with the ones described in [69].

General procedure A for the synthesis of compounds 8a–8i and 9a–9h

To a suspension of 0.735 g 2-cyanobenzoic acid (**5**, 5 mmol) in 50 cm^3 DCM 1.4 cm^3 TEA (10 mmol), methyl ester L-α-amino acid hydrochloride (5 mmol), and 0.675 g HOBt (5 mmol) were added. The mixture was stirred at 0 °C and 0.970 g EDCI (5.05 mmol) was added. Then, the mixture was stirred at room temperature overnight. The precipitate was filtered off and the filtrate was evaporated under reduced pressure. The residue was diluted with 50 cm^3 DCM, washed with solution of 0.1 N HCl (3 × 25 cm^3), 15 cm^3 brine, then dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (gradient elution of ethyl acetate/petroleum ether 0:100–70:30) to afford the desired products as separate fractions of **9a–9h**, then **8a–8i**.

Methyl 2-[[(2-cyanophenyl)carbonyl]amino]acetate (8a, $C_{11}H_{13}N_3O_4$) It was prepared according to General procedure A (0.807 g, 74%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–70:30, R_f = 0.32) as a white powder. M.p.: 112–113 °C; ^1H NMR (300 MHz, $CDCl_3$): δ = 3.74 (s, 3H, CH_3O), 4.21 (d, J = 5.1 Hz, 2H, CH_2), 7.13 (br s, 1H, NH), 7.56 (dd, J_1 = 7.8 Hz, J_2 = 7.8 Hz, 4-H_{Ar}), 7.64 (dd, J_1 = 7.8 Hz, J_2 = 7.8 Hz, 5-H_{Ar}), 7.72 (d, J = 7.8 Hz, 1H, 3-H_{Ar}), 7.78 (d, J = 7.8 Hz, 1H, 6-H_{Ar}) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 42.4, 53.1, 111.6, 118.0, 129.0, 131.9, 133.4, 134.9, 138.1, 166.0, 170.5 ppm; IR (KBr): $\bar{\nu}$ = 3269, 2227 (CN), 1749, 1648, 1538 cm^{-1}; LC/MS: *m/z* (%) = 219 (100, $[M+H]^+$).

Methyl (2*S*)-2-[[(2-cyanophenyl)carbonyl]amino]propanoate (8b, $C_{12}H_{12}N_2O_3$) It was prepared according to General procedure A (0.928 g, 80%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–60:40, R_f = 0.31) as a white powder. M.p.: 96–97 °C; ^1H NMR (300 MHz, $CDCl_3$): δ = 1.56 (d, J = 7.2 Hz, 3H, CH_3), 3.79 (s, 3H, CH_3O), 4.82 (pseudo quintet, J = 7.2 Hz, 1H, $C_\alpha H$), 6.88 (br s, 1H, NH), 7.58 (dd, J_1 = 7.5 Hz, J_2 = 7.5 Hz, 1H, 4-H_{Ar}), 7.66 (dd, J_1 = 7.5 Hz, J_2 = 7.5 Hz, 1H, 5-H_{Ar}), 7.75 (d, J = 7.5 Hz, 1H, 3-H_{Ar}), 7.81 (d, J = 7.5 Hz, 1H, 6-H_{Ar}) ppm; ^{13}C NMR (75 MHz $CDCl_3$,): δ = 18.4, 49.1, 52.8, 111.0, 117.5, 128.7, 131.4, 133.0, 134.3, 138.0, 164.7, 173.2 ppm; IR (KBr): $\bar{\nu}$ = 3298, 2933, 2225 (CN), 1742, 1645, 1591, 1536 cm^{-1}; LC/MS: *m/z* (%) = 233 (100, $[M+H]^+$).

Methyl (2*S*)-2-[[(2-cyanophenyl)carbonyl]amino]-3-methylbutanoate (8c, $C_{14}H_{16}N_2O_3$) It was prepared according to General procedure A (1.105 g, 85%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–60:40, R_f = 0.53) as a white powder. M.p.: 77–78 °C; ^1H NMR (300 MHz, $CDCl_3$): δ = 1.02 (d, J = 6.9 Hz, 3H, $CHC\underline{H}_3$), 1.07 (d, J = 6.9 Hz, 3H, $CHC\underline{H}_3$), 2.28–2.38 (m, 1H, $C\underline{H}CH_3$), 3.79 (s, 3H, CH_3O), 4.81 (dd, J_1 = 8.4 Hz, J_2 = 8.7 Hz, 1H, $C_\alpha H$), 6.77 (d, J = 7.5 Hz, 1H, NH), 7.59 (dd, J_1 = 7.5 Hz, J_2 = 7.8 Hz, J_m = 1.2 Hz, 1H, 4-H_{Ar}), 7.68 (dd, J = 7.8 Hz, J_2 = 7.5 Hz, J_m = 1.2 Hz, 1H, 5-H_{Ar}), 7.78 (d, J = 7.8 Hz, 1H, 3-H_{Ar}), 7.83 (d, J = 7.8 Hz, 1H, 6-H_{Ar}) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.6, 19.7, 32.2, 53.0, 58.7, 111.2, 118.2, 129.6, 131.9, 133.6, 134.8, 138.8, 165.7, 172.8 ppm; IR (KBr): $\bar{\nu}$ = 3271, 2952, 2223 (CN), 1731, 1644, 1591, 1540 cm^{-1}; LC/MS: *m/z* (%) = 261 (100, $[M+H]^+$).

Methyl (2*S*)-2-[[(2-cyanophenyl)carbonyl]amino]-4-methylpentanoate (8d, $C_{15}H_{18}N_2O_3$) It was prepared according to General procedure A (1.248 g, 91%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–60:40, R_f = 0.53) as a white powder. M.p.: 91–92 °C; ^1H NMR (300 MHz, $CDCl_3$): δ = 0.99 and 1.01 (2d, J = 6.6 and 7.2 Hz, 6H, $2CH_3$), 1.68–1.86 [m, 3H, CH_2 + $C\underline{H}(CH_3)_2$], 3.79 (s, 3H, CH_3O), 4.85–4.92 (m, 1H, $C_\alpha H$), 6.64 (d, J = 6.9 Hz, 1H, NH), 7.59 (dd, J_1 = 7.5 Hz, J_2 = 7.5 Hz, 1H, 4-H_{Ar}), 7.66 (dd, J_1 = 7.8 Hz, J_2 = 7.5 Hz, 1H, 5-H_{Ar}), 7.77 (d, J = 7.8 Hz, 1H, 3-H_{Ar}), 7.84 (d, J = 7.8 Hz, 1H, 6-H_{Ar}) ppm; ^{13}C NMR (100 MHz $CDCl_3$,): δ = 22.0, 22.9, 25.0, 41.5, 51.6, 52.6, 110.8, 117.5, 128.7, 131.3, 132.9, 134.2, 137.8, 165.0, 173.3 ppm; IR (KBr): $\bar{\nu}$ = 3288, 2954, 2227 (CN), 1738, 1641, 1535 cm^{-1}; LC/MS: *m/z* (%) = 275 (100, $[M+H]^+$).

Methyl (2*S*)-2-[[(2-cyanophenyl)carbonyl]amino]-4-(methylsulfanyl)butanoate (8e, $C_{14}H_{16}N_2O_3S$) It was prepared according to General procedure A (1.212 g, 83%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–60:40, R_f = 0.38) as a yellowish white powder. M.p.: 90–91 °C; ^1H NMR (300 MHz, $CDCl_3$): δ = 2.09 (s, 3H, CH_3S), 2.10–2.15 (m, 1H, $CHC\underline{H}_2$), 2.26–2.31 (m, 1H, $CHC\underline{H}_2$), 2.58–2.61 (m, 2H, CH_2S), 3.77 (s, 3H, CH_3O), 4.90–4.95 (m, 1H, CH), 7.15 (br s, 1H, NH), 7.57–7.82 (m, 4H, 3-6-H_{Ar}) ppm; ^{13}C NMR

(75 MHz, $CDCl_3$): δ = 16.1, 30.6, 31.9, 53.0, 53.3, 111.4, 118.0, 129.2, 131.9, 133.4, 134.7, 138.3, 165.6, 172.6 ppm; IR (KBr): $\bar{\nu}$ = 3473, 3429, 3313, 2958, 2919, 2228 (CN), 1753, 1645, 1593, 1578, 1535 cm^{-1}; LC/MS: *m/z* (%) = 293 (100, $[M+H]^+$).

Methyl (2*S*)-2-[[(2-cyanophenyl)carbonyl]amino]-3-phenylpropanoate (8f, $C_{18}H_{16}N_2O_3$) It was prepared according to General procedure A (1.324 g, 86%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–60:40, R_f = 0.47) as a white powder. M.p.: 126–127 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.23–3.40 (m, 2H, CH_2), 3.80 (s, 3H, CH_3O), 5.09–5.15 (m, 1H, $C_\alpha H$), 6.64 (d, J = 6.3 Hz, 1H, NH), 7.18 (d, J = 6.6 Hz,1H, H_{Ar}), 7.25–7.32 (m, 5H, H_{Ph}), 7.57–7.66 (m, 3H, 3-5-H_{Ar}), 7.79 (d, J = 7.5 Hz, 1H, 6-H_{Ar}) ppm; ^{13}C NMR (75 MHz $CDCl_3$): δ = 38.3, 53.2, 54.6, 111.7, 117.9, 127.9, 128.9, 129.3 ($2C_{Ph}$-3, 5), 130.0 ($2C_{Ph}$-2, 6), 131.9, 133.4, 135.0, 136.2, 138.3, 165.3, 172.2 ppm; IR (KBr): $\bar{\nu}$ = 3303, 2227 (CN), 1739, 1676, 1639, 1590, 1573, 1525 cm^{-1}; LC/MS: *m/z* (%) = 309 (100, $[M+H]^+$).

Methyl (2*S*)-2-[[(2-cyanophenyl)carbonyl]amino]-3-(1*H*-indol-3-yl)propanoate (8g, $C_{20}H_{17}N_3O_3$) It was prepared according to General procedure A (1.596 g, 92%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–60:40, R_f = 0.28) as a white powder. M.p.: 131–132 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.37–3.52 (m, 2H, CH_2), 3.71 (s, 3H, CH_3O), 5.09–5.15 (m, 1H, $C_\alpha H$), 6.88 (d, J = 4.5 Hz, 1H, CONH), 7.03–7.07 (m, 2H, H_{Ar}), 7.13 (dd, J_1 = 7.8 Hz, J_2 = 7.2 Hz, 1H, H_{Ar}), 7.32 (d, J = 7.8 Hz, 1H, H_{Ind}(2)), 7.43–7.47 (m, 3H, H_{Ar}), 7.53 (d, J = 7.8 Hz, 1H, H_{Ind}), 7.65 (dd, J_1 = 4.5 Hz, J_2 = 6.3 Hz, 1H, H_{Ar}), 8.74 (s, 1H, NH_{Ind}) ppm; ^{13}C NMR (75 MHz, $CDCl_3$,): δ = 27.9, 53.1, 54.4, 109.7, 111.5, 112.1, 118.1, 118.9, 120.0, 122.6, 124.0, 128.1, 128.6, 131.7, 133.2, 134.8, 136.8, 138.1, 165.6, 172.6 ppm; IR (KBr): $\bar{\nu}$ = 3407, 3361, 2228 (CN), 1739 (C=O), 1659 (C–N), 1623, 1593, 1574, 1530 cm^{-1}; LC/MS: *m/z* (%) = 348 (100, $[M+H]^+$).

Dimethyl (2*S*)-2-[[(2-cyanophenyl)carbonyl]amino]pentanedioate (8h, $C_{15}H_{16}N_3O_5$) It was prepared according to General procedure A (1.277 g, 84%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–60:40, R_f = 0.39) as a white powder. M.p.: 84–85 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.08–2.21 (m, 1H, $CHC\underline{H}_2$), 2.27–2.39 (m, 1H, $CHC\underline{H}_2$), 2.43-2.60 (m, 2H, CH_2CO), 3.65 (s, 3H, $CH_3O_2CCH_2$), 3.77 (s, 3H, CH_3O_2CCH), 4.79-4.86 (m, 1H, $C_\alpha H$), 7.18 (d, J = 6.9 Hz, 1H, NH), 7.58 (dd, J_1 = 7.5 Hz, J_2 = 7.8 Hz, 1H, 4-H_{Ar}), 7.66 (dd, J_1 = 7.5 Hz, J_2 = 7.5 Hz, 1H, 5-H_{Ar}), 7.75 (d, J = 7.5 Hz, 1H, 3-H_{Ar}), 7.77 (d, J = 7.8 Hz, 1H, 6-H_{Ar}) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 27.6, 30.7, 52.5, 53.2, 53.4, 111.6, 118.0, 129.1, 132.0, 133.4, 134.9, 138.3, 165.7, 172.5, 174.1 ppm; IR (KBr): $\bar{\nu}$ = 3459, 3317, 2230 (CN), 1753, 1737, 1686, 1645, 1594, 1578, 1530 cm^{-1}; LC/MS: *m/z* (%) = 305 (95, $[M+H]^+$).

Methyl *cis/trans*-(2*S*)-1-[(2-cyanophenyl)carbonyl]pyrrolidine-2-carboxylate (8i, $C_{14}H_{14}N_2O_3$) It was prepared according to General procedure A (1.226 g, 95%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–70:30, R_f = 0.34) as colourless crystals. M.p.: 87–88 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.89–2.14 (m, 4H, CH_2), 2.29–2.37 (m, 1.3H, CH_2), 3.36–3.40 (m, 1H, $C\underline{H}_2C_\alpha H$-$Pro_{trans}$), 3.45–3.49 (m, 1H, $C\underline{H}_2C_\alpha H$-$Pro_{trans}$), 3.53 (s, 0.87H, OCH_{3cis}), 3.76 (s, 3H, OCH_{3trans}), 3.80–3.83 (m, 0.62H, $C\underline{H}_2C_\alpha H$-$Pro_{cis}$), 4.23–4.25 (m, 0.29H, $C_\alpha H$-Pro_{cis}), 4.68–4.71 (m, 1H, $C_\alpha H$-Pro_{trans}), 7.40 (d, J = 7.5 Hz, 0.29H, H-Ar_{cis}), 7.46–7.71 (m, 5H, 3-6-H_{Ar}) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ *trans*(*cis*) = (22.8) 25.0, 29.5 (31.4), (46.7) 49.0, 52.5 (52.6), 58.0 (60.9), (110.02) 110.04, (116.8) 116.9, 127.65 (127.68), (129.9) 130.0, (132.9) 133.0, (133.1) 133.3, 140.4 (140.6), 166.2 (166.6), (172.0) 172.1 ppm; IR (KBr): $\bar{\nu}$ = 2953, 2227 (CN), 1735, 1624, 1591, 1490, 1414 cm^{-1}; LC/MS: *m/z* (%) = 259 (100, $[M+H]^+$).

Methyl 2-(1-imino-3-oxo-1,3-dihydro-2*H*-isoindol-2-yl)acetate (9a) It was prepared according to General procedure A (0.164 g, 15%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–70:30, R_f = 0.51) as colourless crystals. ^{1}H NMR and ^{13}C NMR spectra were found to be identical with the ones described in Ref. [43]; IR (KBr): $\bar{\nu}$ = 3449, 3287, 1741, 1728, 1662, 1621, 1472, 1437 cm^{-1}; LC/MS: *m/z* (%) = 219 (100, $[M+H]^+$).

Methyl (2*S*)-2-(1-imino-3-oxo-1,3-dihydro-2*H*-isoindol-2-yl)propanoate (9b) It was prepared according to General procedure A (0.105 g, 9%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–60:40, R_f = 0.52) as a colourless crystals. ^{1}H NMR and ^{13}C NMR spectra were found to be identical with the ones described in Ref. [43]; IR (KBr): $\bar{\nu}$ = 3279, 2915, 1735, 1717, 1652, 1420 cm^{-1}; LC/MS: *m/z* (%) = 233 (100, $[M+H]^+$).

Methyl (2*S*)-2-(1-imino-3-oxo-1,3-dihydro-2*H*-isoindol-2-yl)-3-methylbutanoate (9c) It was prepared according to General procedure A (0.078 g, 6%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–60:40, R_f = 0.67) as a white powder. ^{1}H NMR and ^{13}C NMR spectra were found to be identical with the ones described in Ref. [43]. IR (KBr): $\bar{\nu}$ = 3437, 3403, 3292, 2965, 1772, 1741, 1728, 1687, 1660, 1470, 1415, 1390 cm^{-1}; LC/MS: *m/z* (%) = 261 (100, $[M+H]^+$).

Methyl (2*S*)-2-(1-imino-3-oxo-1,3-dihydro-2*H*-isoindol-2-yl)-4-methylpentanoate (9d) It was prepared according to General procedure A (0.055 g, 4%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–60:40, R_f = 0.72) as a colourless viscous substance. ^{1}H NMR and ^{13}C NMR spectra were found to be identical with the ones described in Ref. [43]; IR (KBr): $\bar{\nu}$ = 3547, 3464, 3371, 3289, 2958, 2875, 1732, 1652, 1472, 1416 cm^{-1}; LC/MS: *m/z* (%) = 275 (100, [M+H]$^+$).

Methyl (2*S*)-2-(1-imino-3-oxo-1,3-dihydro-2*H*-isoindol-2-yl)-4-(methylsulfanyl)butanoate (9e) It was prepared according to General procedure A (0.102 g, 7%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–60:40, R_f = 0.56) as a yellowish powder. M.p.: 79–80 °C; ^{1}H NMR and ^{13}C NMR spectra were found to be identical with the ones described in Ref. [43]; IR (KBr): $\bar{\nu}$ = 3450, 3294, 2914, 1772, 1743, 1733, 1661, 1444, 1415 cm^{-1}; LC/MS: *m/z* (%) = 293 (100, [M+H]$^+$).

Methyl (2*S*)-2-(1-imino-3-oxo-1,3-dihydro-2*H*-isoindol-2-yl)-3-phenylpropanoate (9f) It was prepared according to General procedure A (0.046 g, 3%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–60:40, R_f = 0.66) as a yellowish viscous substance. ^{1}H NMR and ^{13}C NMR spectra were found to be identical with the ones described in Ref. [43]; LC/MS: *m/z* (%) = 309 (100, [M + H]$^+$).

General procedure B for the synthesis of compounds 10a–10i

To a stirred solution of 0.520 g hydroxylamine hydrochloride (7.5 mmol) in 20 cm^3 methanol 1.05 cm^3 TEA (7.5 mmol) was added at 0 °C. After 15 min appropriate 2-cyanobenzamide **8** (5 mmol) was added to this solution. The reaction mixture was stirred from 0 °C to ambient temperature until the TLC showed the absence of starting material. After that the clear solution was concentrated to ~ 5 cm^3 under reduced pressure at ambient temperature, diluted with 15 cm^3 brine and then extracted with 5 × 25 cm^3 ethyl acetate. The combined parts of the extract were dried over $MgSO_4$ and concentrated under reduced pressure at ambient temperature. The residue was then purified by column chromatography (ethyl acetate/petroleum ether 7:3) to afford the desired product as a white solid.

Methyl 2-[[[2-(*N*′-hydroxycarbamimidoyl)phenyl]carbonyl]-amino]acetate (10a, $C_{11}H_{13}N_3O_4$) It was prepared according to General procedure B (0.791 g, 72%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–90:10, R_f = 0.27) as a white powder. M.p.: 126–128 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.67 (s, 3H, CH_3O), 3.95 (d, J = 5.2 Hz, 2H, CH_2), 5.64 (s, 2H, NH_2), 7.46–7.48 (m, 4H, 3-6-H_{Ar}), 8.62 (br m, 1H, NH), 9.52 (s, 1H, OH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 41.6, 52.3, 128.3, 129.1, 129.5, 129.9, 132.8, 136.5, 152.1, 169.6, 170.8 ppm; IR (KBr): $\bar{\nu}$ = 3416, 3362, 1738, 1631, 1539 cm^{-1}; LC/MS: *m/z* (%) = 252 (100, [M+H]$^+$).

Methyl (2*S*)-2-[[[2-(*N*′-hydroxycarbamimidoyl)phenyl]carbonyl]amino]propanoate (10b, $C_{12}H_{15}N_3O_4$) It was prepared according to General procedure B (0.994 g, 75%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–90:10, R_f = 0.31) as a white powder. M.p.:134–136 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.34 (d, J = 7.2 Hz, 3H, CH_3), 3.66 (s, 3H, CH_3O), 4.37–4.47 (pseudo quintet, J = 7.2 Hz, 1H, C_αH), 5.66 (s, 2H, NH_2), 7.45–7.48 (m, 4H, 3-6-H_{Ar}), 8.51 (d, J = 6.9 Hz, NH), 9.51 (s, 1H, OH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 17.5, 48.6, 52.6, 128.5, 129.2, 129.6, 130.0, 133.0, 136.5, 152.2, 168.8, 173.7 ppm; IR (KBr): $\bar{\nu}$ = 3486, 3439, 3332, 3208, 3034, 2940, 2888, 1733, 1638, 1601, 1586, 1563 cm^{-1}; LC/MS: *m/z* (%) = 266 (100, [M+H]$^+$).

Methyl (2*S*)-2-[[[2-(*N*′-hydroxycarbamimidoyl)phenyl]carbonyl]amino]-3-methylbutanoate (10c, $C_{14}H_{19}N_3O_4$) It was prepared according to General procedure B (1.216 g, 83%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–90:10, R_f = 0.50) as colourless crystals. M.p.: 121–122 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.95 (d, J = 6.9 Hz, 3H, CH_3), 0.99 (d, J = 6.9 Hz, 3H, CH_3), 2.15–2.26 (m, 1H, C<u>H</u>$(CH_3)_2$), 3.74 (s, 3H, CH_3O), 4.67 (dd, J_1 = 8.4 Hz, J_2 = 8.4 Hz, C_αH), 5.07 (s, 2H, NH_2), 7.21 (d, J = 8.4 Hz, 1H, NH), 7.43–7.47 (m, 3H, 3-5-H_{Ar}), 7.64–7.66 (m, 1H, 6-H_{Ar}) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.7, 19.6, 31.9, 52.9, 58.8, 129.5, 130.1, 130.5, 131.0, 131.2, 136.1, 153.7, 169.2, 173.0 ppm; IR (KBr): $\bar{\nu}$ = 3439, 3325, 2956, 1732, 1623, 1598, 1541 cm^{-1}; LC/MS: *m/z* (%) = 294 (100, [M+H]$^+$).

Methyl (2*S*)-2-[[[2-(*N*′-hydroxycarbamimidoyl)phenyl]carbonyl]amino]-4-methylpentanoate (10d, $C_{15}H_{21}N_3O_4$) It was prepared according to General procedure B (1.366 g, 89%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–90:10, R_f = 0.53) as a white powder. M.p.: 129–130 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.90 (2d, J = 6.9 Hz, 6H, $2CH_3$), 1.56-1.76 (m, 3H, $CH(CH_3)_2$ + CH_2), 3.66 (s, 3H, CH_3O), 4.38-4.46 (m, 1H, C_αH), 5.64 (s, 2H, NH_2), 7.46 (s, 4H, 3-6-H_{Ar}), 8.50 (d, J = 7.5 Hz, 1H, NH), 9.50 (s, 1H, OH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 21.9, 23.4, 24.6, 40.3, 51.3, 52.4, 128.6, 129.1, 129.7, 130.0, 132.8, 136.2,

152.3, 168.8, 173.4 ppm; IR (KBr): $\bar{\nu}$ = 3457, 3399, 3335, 2953, 1719, 1642, 1518 cm^{-1}; LC/MS: *m/z* (%) = 308 (100, $[M+H]^+$).

Methyl (2*S*)-2-[[[2-(*N*′-hydroxycarbamimidoyl)phenyl]carbonyl]amino]-4-(methylsulfanyl)butanoate (10e, $C_{14}H_{19}N_3O_4S$) It was prepared according to General procedure B (1.333 g, 82%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–90:10, R_f = 0.43) as a pale yellow powder. M.p.: 88–90 °C; ^{1}H NMR [300 MHz, $(CD_3)_2CO$]: δ = 2.05–2.20 (m, 5H, CH_3S, $CHCH_2$), 2.62–2.68 (m, 2H, CH_2S), 3.71 (s, 3H, CH_3O), 4.70–4.78 (m, 1H, $C_\alpha H$), 5.60 (s, 2H, NH_2), 7.45–7.49 (m, 3H, 3-5-H_{Ar}), 7.60–7.63 (m, 1H, 6-H_{Ar}), 8.01 (d, J = 7.8 Hz, 1H, NH), 9.01 (br s, 1H, OH) ppm; ^{13}C NMR (75 MHz, $(CD_3)_2CO$): δ = 15.3, 30.8, 32.3, 52.6, 52.8, 129.6, 129.9, 130.3, 130.7, 132.9, 136.6, 153.5, 169.1, 173.0 ppm; IR (KBr): $\bar{\nu}$ = 3473, 3440, 3365, 3314, 3265, 1736, 1637, 1601, 1579, 1550, 1438, 1382; LC/MS: *m/z* (%) = 326 (100, $[M+H]^+$).

Methyl (2*S*)-2-[[[2-(*N*′-hydroxycarbamimidoyl)phenyl]carbonyl]amino]-3-phenylpropanoate (10f, $C_{18}H_{19}N_3O_4$) It was prepared according to General procedure B (1.500 g, 88%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–90:10, R_f = 0.49) as a white powder. M.p.: 119–121 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 3.12–3.26 (m, 2H, CH_2), 3.72 (s, 3H, CH_3O), 4.96 (s, 2H, NH_2), 5.01–5.08 (m, 1H, $C_\alpha H$), 7.17–7.57 (m, 10H, H_{Ar} + NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 37.4, 52.5, 54.7, 127.1, 128.5, 128.9 (C_{Ph}-3 + C_{Ph}-5), 129.1, 129.7 (C_{Ar}-5 + C_{Ph}-2 + C_{Ph}-6), 130.0, 132.8, 136.1, 137.7, 152.3, 168.8, 172.3 ppm; IR (KBr): $\bar{\nu}$ = 3432, 3285, 1748, 1624, 1591, 1552 cm^{-1}; LC/MS: *m/z* (%) = 342 (100, $[M+H]^+$).

Methyl (2*S*)-2-[[[2-(*N*′-hydroxycarbamimidoyl)phenyl]carbonyl]amino]-3-(1*H*-indol-3-yl)propanoate (10g, $C_{20}H_{20}N_4O_4$) It was prepared according to General procedure B (1.767 g, 93%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–90:10, R_f = 0.34) as a yellowish white powder. M.p.: 108–110 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.15–3.24 (m, 2H, CH_2), 3.61 (s, 3H, CH_3O), 4.66–4.73 (m, 1H, $C_\alpha H$), 5.66 (s, 2H, NH_2) 7.02 (dd, J_1 = 7.2 Hz, J_2 = 7.5 Hz, 1H, C_6H_{Ind}), 7.10 (dd, J_1= 7.2 Hz, J_2 = 7.5 Hz, 1H, C_5H_{Ind}), 7.27 (s, 1H, $C_\alpha H_{Ind}$), 7.37 (d, J = 7.8 Hz, 1H, C_7H_{Ind}), 7.41–7.48 (m, 4H, 4-7-H), 7.55 (d, J = 7.8 Hz, 1H, C_4H_{Ind}), 8.58 (d, J = 7.5 Hz, 1H, CONH), 9.57 (s, 1H, OH), 10.84 (s, 1H, NH_{Ind}) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 27.1, 51.9, 53.7, 109.4, 111.5, 118.1, 118.4, 121.0, 123.9, 127.1, 128.1, 128.6, 129.2, 129.5, 132.3, 135.7, 136.1, 151.8, 168.2, 172.2 ppm; IR (KBr): $\bar{\nu}$ = 3375, 3059, 1736, 1642, 1599, 1524 cm^{-1}; LC/MS: *m/z* (%) = 381 (100, $[M+H]^+$).

Dimethyl (2*S*)-2-[[[2-(*N*′-hydroxycarbamimidoyl)phenyl]carbonyl]amino]pentanedioate (10h, $C_{15}H_{19}N_3O_6$) It was prepared according to General procedure B (1.365 g, 81%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–90:10, R_f = 0.45) as a white powder. M.p.: 97–99 °C; ^{1}H NMR (300 MHz, CD_3CN): δ = 1.92–2.02 (m, 1H, $CHCH_2$), 2.12–2.23 (m, 1H, $CHCH_2$), 2.48–2.51 (m, 2H, CH_2CO), 3.65 (s, 3H, $CH_2CO_2CH_3$), 3.72 (s, 3H, $C_\alpha CO_2CH_3$), 4.54–4.62 (m, 1H, $C_\alpha H$), 5.26 (s, 2H, NH_2), 7.47–7.56 (m, 5H, 3-6-H_{Ar} + NH) ppm; ^{13}C NMR (75 MHz, CD_3CN): δ = 27.7, 30.8, 52.4, 53.06, 53.12, 129.3, 130.3, 130.4, 131.1, 132.5, 136.7, 153.7, 169.7, 173.1, 174.5 ppm; IR (KBr): $\bar{\nu}$ = 3464, 3390, 3321, 1735, 1716, 1642, 1599, 1578, 1535 cm^{-1}; LC/MS: *m/z* (%) = 338 (100, $[M+H]^+$).

Methyl *cis/trans*-(2*S*)-1-[[2-(*N*′-hydroxycarbamimidoyl)-phenyl]carbonyl]pyrrolidine-2-carboxylate (10i, $C_{14}H_{17}N_3O_4$) It was prepared according to the General procedure B (1.339 g, 92%). The product was isolated by column chromatography (gradient elution of EA/PE 0:100–90:10, R_f = 0.23) as a colourless viscous substance. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.80–2.01 (m, 3.2H, H-Pro), 2.22–2.29 (m, 1.1H, H-Pro), 3.21–3.31 (m, 1.5H, NCH_2-Pro), 3.47 (s, 0.8H, CH_3O) 3.68–3.74 (m, 2.8H, CH_3O + H-Pro), 4.17–4.20 (m, 0.23H, $C_\alpha H$), 4.57–4.62 (m, 0.77H, $C_\alpha H$), 5.17 (s, 2H, NH_2), 7.20–7.56 (m, 4.3H, H_{Ar}-3-6) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = (23.4) 25.3, 30.1 (31.5), (46.9) 49.5, (52.8) 52.9, 59.2 (61.4), 127.5 (127.6), (129.1) 129.2, (129.9) 130.0, 130.1, 130.3, (136.3) 136.4, (152.7) 152.9, 170.2 (170.5), 173.1 ppm; IR (KBr): $\bar{\nu}$ = 3339, 2952, 1733, 1613, 1595, 1499, 1451, 1420 cm^{-1}; LC/MS: *m/z* (%) = 292 (100, $[M+H]^+$).

General procedure C for the synthesis of 3-(arylimino)-2,3-dihydro-1*H*-isoindol-1-ones 15a, 15b [61]

To the solution of 0.292 g 3-imino-2,3-dihydro-1*H*-isoindol-1-one (**14**, 2 mmol) in a mixture of 10 cm^3 ethanol and 0.4 cm^3 acetic acid appropriate amount of arylamine (6.5 mmol) was added and reaction mixture was heated under reflux until the TLC showed the absence of starting compound **14**. The obtained orange or yellow solution was cooled and poured into 20 cm^3 water to give an ochre or yellow precipitate. This substance was filtered by suction, washed with distilled water (3 × 5 cm^3) and dried in the air. After recrystallization from ethanol the obtained product **15** was used in the next step.

(3*Z*,*E*)-3-[(2-Methylphenyl)imino]-2,3-dihydro-1*H*-isoindol-1-one (15a, $C_{15}H_{12}N_2O$) It was prepared according to the General procedure C (0.42 g, 89%, *Z*/*E* = 83:17). The product was isolated after recrystallization in ethanol as yellow crystals. M.p.: 154–155 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.16 (s, 0.52H, $CH_{3,E}$), 2.23 (s, 2.48H, $CH_{3,Z}$), 6.60 (d, J = 7.8 Hz, 0.17H, 4-H_E), 6.88–6.90 (m, 1H, 3′-H_{tol}), 7.09–7.29 (m, 3H, 4′-6′H_{tol}), 7.37 (dd, J_1 = 7.8 Hz, J_2= 7.5 Hz, 0.17H, 5-H_E) 7.48 (bs, 1H, NH), 7.59 (dd, J_1 = 7.5 Hz, J_2 = 7.8 Hz, 0.17H, 6-H_E), 7.68–7.80 (m, 1.66H, 5,6-H_Z), 7.87–7.90 (m, 1H, 7-H_{Z+E}), 8.10 (d, 0.83H, J = 7.8 Hz, 4-H_Z) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.8 (17.9) (CH_3), 119.7, 122.5, 123.5, (123.7), (124.6), 125.0, (125.3), 126.8, (128.2), 129.6, (130.9), 131.0, 131.5, 132.3, (132.4), (133.2), 133.6, 135.9, 145.5, 148.4, 168.2 ppm; IR (KBr): $\bar{\nu}$ = 3220, 3053, 1736, 1675, 1475, 1348, 1307 cm^{-1}; LC/MS: *m/z* (%) = 237 (100, $[M+H]^+$).

(3*Z*,*E*)-3-[(4-Chlorophenyl)imino]-2,3-dihydro-1*H*-isoindol-1-one (15b) It was prepared according to the General procedure C (0.47 g, 91%). The product was isolated after recrystallization in ethanol as yellow silky needles. M.p.: 219–220 °C; ^{1}H NMR and ^{13}C NMR spectra were found to be identical with the ones described in [62].

General procedure D for the synthesis of compounds 16a–16d [38]

To a solution of appropriate amount of 3-(arylimino)-2,3-dihydro-1*H*-isoindol-1-one **15** (0.75 mmol) in 10 cm^3 dry acetone methyl 2-bromoalkanoate (1.12 mmol), 0.186 g potassium iodide (1.12 mmol), and 0.207 g anhydrous potassium carbonate (1.5 mmol) were added. The obtained mixture was stirred at room temperature until the TLC showed the absence of starting isoindolone **15** (∼ 2–3 days). After that inorganic salts were filtered off and washed with 5 cm^3 acetone. The filtrate was concentrated under reduced pressure and the crude product was purified by column chromatography (EtOAc/petroleum ether 1:1) to afford the desired compound **16**.

Methyl [(1*E*)-1-[(2-methylphenyl)imino]-3-oxo-1,3-dihydro-2*H*-isoindol-2-yl]acetate (16a, $C_{18}H_{16}N_2O_3$) It was prepared according to General procedure D (0.195 g, 84%). The product was isolated by column chromatography (EtOAc/petroleum ether 1:1) as a orange viscous susbtance. TLC: R_f = 0.72; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.06 (s, 3H, CH_3–C_{Ar}), 3.74 (s, 3H, CH_3O), 4.67 (s, 2H, CH_2), 6.63 (d, J = 7.8 Hz, 1H, 4-H), 6.83 (d, J = 7.5 Hz, 1H, H_{Ar}), 7.08 (dd, J_1 = 7.2 Hz, J_2 = 7.2 Hz, 1H, H_{Ar}), 7.17 (dd, J_1 = 7.5 Hz, J_2 = 7.5 Hz, 1H, H_{Ar}), 7.22 (d, J = 7.5 Hz, 1H, H_{Ar}), 7.30 (dd, J_1 = 7.5 Hz, J_2 = 7.5 Hz, 1H, 5-H), 7.51 (dd, J_1 = 7.5 Hz, J_2 = 7.5 Hz, 1H, 6-H), 7.85 (d, J = 7.5 Hz, 1H, 7-H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.2, 40.4, 52.9, 119.7, 124.1, 124.9, 125.5, 127.2, 128.4, 130.4, 131.3, 132.6, 132.7, 133.7, 147.3, 150.4, 167.6, 168.9 ppm; IR (KBr): $\bar{\nu}$ = 3404, 2951, 1736, 1673, 1413, 1215 cm^{-1}; LC/MS: *m/z* (%) = 309 (100, $[M+H]^+$).

Methyl 2-[(1*E*)-1-[(2-methylphenyl)imino]-3-oxo-1,3-dihydro-2*H*-isoindol-2-yl]propanoate (16b, $C_{19}H_{18}N_2O_3$) It was prepared according to General procedure D (0.193 g, 80%). The product was isolated by column chromatography (EtOAc/petroleum ether 1:1) as an orange viscous substance. TLC: R_f = 0.78; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.78 (d, J = 7.2 Hz, 3H, CH_3C_α), 2.10 (s, 3H, CH_3-C_{Ar}), 3.76 (s, 3H, CH_3O), 5.29 (q, J = 7.2 Hz, 1H, $C_\alpha H$), 6.63 (d, J = 7.5 Hz, 1H, 4-H), 6.82 (d, J = 7.5 Hz, 1H, H_{Ar}), 7.11 (dd, J_1 = 7.2 Hz, J_2 = 7.5 Hz, 1H, H_{Ar}), 7.20 (dd, J_1 = 7.5 Hz, J_2 = 7.8 Hz, 1H, H_{Ar}), 7.25 (d, J = 7.5 Hz, 1H, H_{Ar}), 7.32 (dd, J_1 = 7.5 Hz, J_2 = 7.5 Hz, 1H, 5-H), 7.54 (dd, J_1 = 7.5 Hz, J_2 = 7.5 Hz, 1H, 6-H), 7.87 (d, J = 7.5 Hz, 1H, 7-H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 15.8, 18.4, 48.6, 53.1, 119.8, 124.2, 125.0, 125.6, 127.3, 128.4, 130.4, 131.4, 132.7, 132.8, 133.7, 147.6, 149.9, 167.5, 171.5 ppm; IR (KBr): $\bar{\nu}$ = 3398, 2995, 2949, 1750, 1670, 1393, 1226 cm^{-1}; LC/MS: *m/z* (%) = 323 (100, $[M+H]^+$).

Methyl [(1*E*)-1-[(4-chlorophenyl)imino]-3-oxo-1,3-dihydro-2*H*-isoindol-2-yl]acetate (16c, $C_{17}H_{13}ClN_2O_3$) It was prepared according to General procedure D (0.225 g, 93%). The product was isolated by column chromatography (EtOAc/petroleum ether 1:1) as yellow crystals. M.p.: 111–112 °C; R_f = 0.73; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.77 (s, 3H, CH_3O), 4.63 (s, 2H, CH_2), 6.77 (d, J = 7.8 Hz, 1H, 4-H), 6.90 (d, J = 8.7 Hz, 2H, 2′-H + 6′-H), 7.33–7.41 (m, 3H, 3′-H + 5′-H + 5-H), 7.57 (dd, J_1 = 7.5 Hz, J_2 = 7.5 Hz, 1H, 6-H), 7.88 (d, J = 7.5 Hz, 1H, 7-H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 40.5, 53.1, 122.0, 124.5, 126.1, 130.1, 130.2 (2C), 130.3, 133.0, 133.6, 147.4, 151.2, 167.7, 168.9 ppm; IR (KBr): $\bar{\nu}$ = 3394, 2941, 1759, 1731, 1669, 1420, 1211 cm^{-1}; LC/MS: *m/z* (%) = 329 (100, $[M+H]^+$).

Methyl 2-[(1*E*)-1-[(4-chlorophenyl)imino]-3-oxo-1,3-dihydro-2*H*-isoindol-2-yl]propanoate (16d, $C_{18}H_{15}ClN_2O_3$) It was prepared according to General procedure D (203 mg, 79%). The product was isolated by column chromatography (EtOAc/petroleum ether 1:1) as yellow crystals. M.p.: 183–184 °C; TLC: R_f = 0.75; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.75 (d, J = 7.2 Hz, 3H, CH_3C_α), 3.76 (s, 3H, CH_3O), 5.24 (q, J = 7.2 Hz, 1H, $C_\alpha H$), 6.77 (d, J = 7.5 Hz, 1H, 4-H), 6.90 (d, J = 8.4 Hz, 2H, 2′-H, 6′-H), 7.35-7.41 (m, 3H, 3′-H + 5′-H + 5-H), 7.57 (dd, J_1 = 7.5 Hz, J_2 = 7.5 Hz, 1H, 6-H), 7.88 (d, J = 7.5 Hz,

1H, 7-H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 15.7, 48.6, 53.0, 121.8, 124.3, 125.9, 129.9, 130.0 (2C), 130.1, 132.8, 133.4, 147.3, 150.5, 167.3, 171.3 ppm; IR (KBr): $\bar{\nu}$ = 3422, 2977, 2940, 2739, 2677, 2492, 1685, 1475, 1398 cm^{-1}; LC/MS: *m/z* (%) = 343 (100, $[M+H]^+$).

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