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Design, synthesis, and preliminary evaluation of 4-(6-(3-nitroguanidino)hexanamido)pyrrolidine derivatives as potential iNOS inhibitors

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Abstract—A series of 4-(6-(3-nitroguanidino)hexanamido)pyrrolidine derivatives were synthesized and evaluated for their abilities to inhibit inducible nitric oxide synthase (iNOS) isoform. All target compounds were prepared in 11 steps from commercially *trans*-4-hydroxy-L-proline. The preliminary pharmacological test showed that three compounds, **17**, **21**, and **30**, have the good potency (IC_{50} = 2.36, 2.68, 2.5 μM, respectively) which are compared to the NOS inhibitor N^G-nitroarginine(*L*-NNA) (IC_{50} = 14.74 μM), and could be used as lead compounds for exploring new iNOS inhibitors in the future.

1. Introduction

Nitric oxide (NO), produced by the enzymatic oxidation of L-arginine by nitric oxide synthase (NOS), plays an important role in many physiological and pathological processes.[1,2] Three isoforms of NOS have been identified to date: iNOS, the inducible isoform found in macrophages and other tissues; eNOS, the constitutive isoform in endothelial cells and functioning in the regulation of blood pressure; and nNOS, the constitutive isoform in neurons. To date, the scientific research suggested that elevated levels of nitric oxide (NO) generated by iNOS are associated with cellular cytotoxicity and tissue damage, which will lead to inflammatory disease. Therefore, the selective iNOS inhibitors have been regarded as potential and effective treatment for inflammatory disease, such as arthritis. The classical NOS inhibitors such as N^G-nitroarginine(*L*-NNA) and N^G-methylarginine (*L*-NMA) are analogues of the endogenous NOS substrate L-arginine. It was reported that 2-iminopyrrolidines had been shown to be potent and selective inhibitors of the human inducible nitric oxide synthase (hiNOS) isoform versus the human endothelial nitric oxide synthase (heNOS).[3,4] Conformationally restricted pyrrolidine-containing dipeptides derived from the dipeptide L-Arg^{NO_2}-L-Dbu-NH_2 showed better potent and selective inhibitory activities (Fig. 1).[5,6] Therefore, the pyrrolidine ring could be used as potential lead scaffold to develop novel selective iNOS inhibitors.

Recently, pyrrolidine derivatives based on L-hydroxyproline scaffold were synthesized and showed better matrix metalloproteinase (MMP) and aminopeptidase N (APN) inhibitory activities in our laboratory.[7,8] In our ongoing work, we will use conformationally restricted pyrrolidine ring as a scaffold to develop selective pyrrolidine inhibitors of the isoforms of iNOS. In order to find the novel pyrrolidine derivatives, we started to optimize this scaffold with following chemical modification: (i) 6-(3-nitroguanidino)hexanoic acid fragment was linked to 4-position of pyrrolidine so as to mimic the structure of *L*-NNA; (ii) keep the free secondary amine in 1-position of pyrrolidine; (iii) using different substituted aromatic ring connected to the nitrogen atoms of amide derived from carboxylic acid of proline. In this study, we reported the preparation and inhibitory activity assays of 4-(6-(3-nitroguanidino)hexanamido)pyrrolidine derivatives.

2. Chemistry

The target compounds were synthesized efficiently following the procedures shown in Scheme 1. Briefly, the

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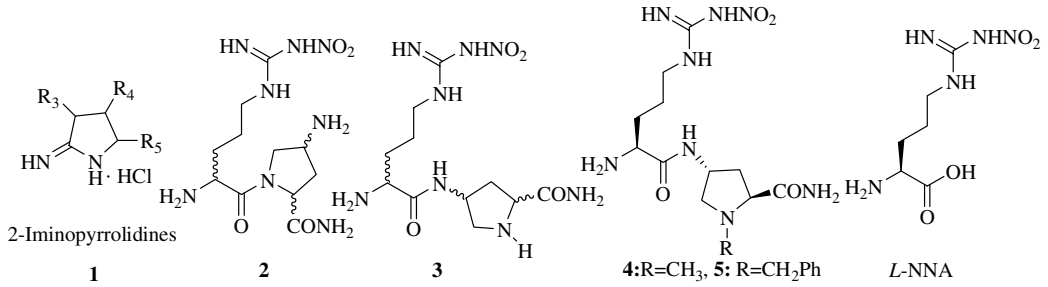
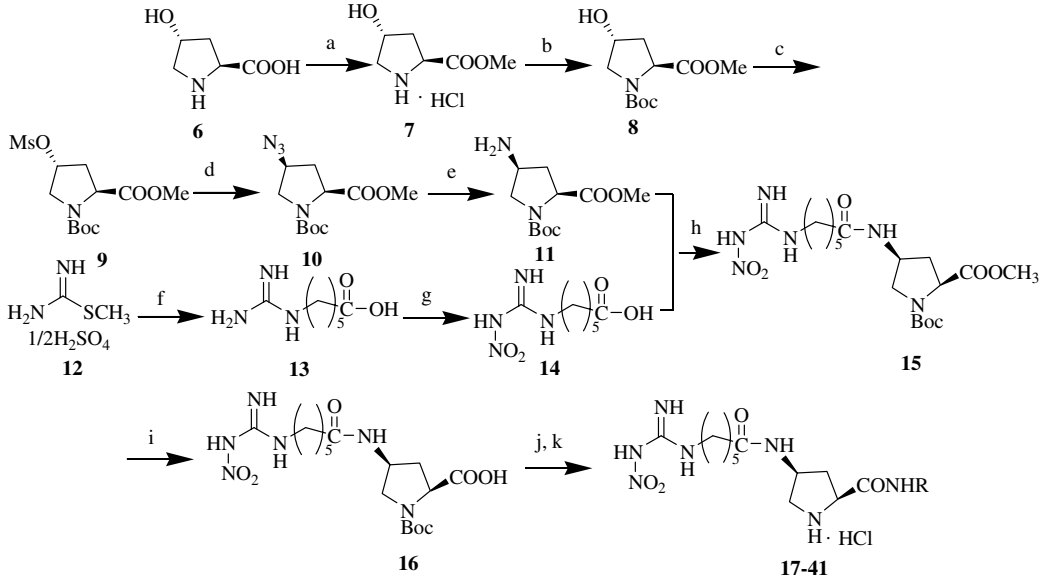


Figure 1. Structures of 2-iminopyrrolidines, pyrrolidine-containing dipeptides, and *L*-NNA.



Scheme 1. Reagents and conditions: (a) MeOH, HCl; (b) $(Boc)_2O$, Et_3N, DCM; (c) MsCl, Et_3N, DCM; (d) NaN_3, DMF, 60 °C; (e) H_2, 5% Pd/$CaCO_3$, MeOH; (f) 3 N NaOH, 6-aminocaproic acid; (g) fuming nitric acid, fuming sulfuric acid (50%), concentrated sulfuric acid; (h) DCC, HOBT, THF; (i) 1 N NaOH, DME; (j) IBCF, THF, NMM, R = substituted phenyl group; (k) HCl/EtOAc.

compound **11** was prepared starting from commercially available *trans*-4-hydroxy-L-proline **6**, followed by the sequential reactions of esterification, *N*-Boc protection, mesylation, S_N 2 displacement with sodium azide, and hydrogenation over 5% Pd/$CaCO_3$. The sulfate salt of 2-methyl-2-thiopseudourea **12** reacted with 6-aminocaproic acid to give compound **13** and then converted to **14** by nitration in fuming nitric acid and 50% fuming sulfuric acid. Acylation of compound **11** with **14** afforded the key intermediate **15**. The target compounds were finally obtained through the reaction sequence including saponification, condensation, and *N*-Boc deprotection from the intermediate **15**.

In the synthesis of the target compounds, we encountered a problem that DCC/HOBT was not suitable for the acylating reaction of amine with compound **16**. Using this system, compound **16** cannot be converted to the target compounds, because of the steric hindrance of Boc group at *N*-position. If compound **16** were converted to its acyl chloride, Boc group in compound **16** would be destroyed. Finally, the target compounds **17**–**41** were obtained by coupling reaction using the mixed anhydride method [isobutylchloroformate (IBCF)/*N*-methylmorpholine (NMM)] successfully.

3. Results and discussion

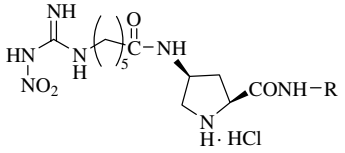
3.1. Structure–activity relationship (in vitro)

Compounds **17**–**41** were evaluated as inhibitors of iNOS. The results of their inhibitory activities (IC_{50}) was reported in Table 1.

Inhibitory activities of **17**–**41** against iNOS:

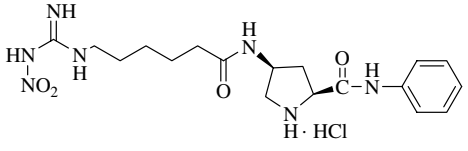
Preliminary result showed that 25 compounds displayed inhibitory activities with IC_{50} value from 2.36 to 162 μM. Compound **17** with phenyl group showed the best inhibitory activity (IC_{50} = 2.36 μM). Compounds **21** and **30** exhibited similar activities (IC_{50} = 2.68, 2.5 μM, respectively) compared with compound **17**. Compounds (**18**, **22**, **25** and **35**) had lower IC_{50} value (7.37–13 μM). Comparing the activities of **18** and **19**, mono-substitution of fluorine at the *ortho*-position in the aromatic ring showed higher affinities than at the *para*-position. On the other hand, di-substitution of fluorine at the 3-, 4-position and 3-, 5-position of the aromatic ring exhibited better potency than at the 2-, 4-position. Substitution of a simple chloro group at the 3-position of the aromatic ring showed moderate po-

Table 1. The structures and in vitro inhibitory activities of compounds against iNOS



Compound	R	iNOS IC_{50} (μM)
17	$-C_6H_5$	2.36
18	*o*-F–C_6H_4	13.1
19	*p*-F–C_6H_4	87
20	2,4-F_2–C_6H_3	139
21	3,4-F_2–C_6H_3	2.68
22	3,5-F_2–C_6H_3	11.7
23	2,3,4-F_3–C_6H_2	36.2
24	*o*-Cl–C_6H_4	108
25	*m*-Cl–C_6H_4	13
26	*p*-Cl–C_6H_4	80
27	2,4-Cl_2–C_6H_3	15.5
28	2,5-Cl_2–C_6H_3	105
29	3,5-Cl_2–C_6H_3	87.3
30	3-Cl-4-CH_3–C_6H_3	2.5
31	*o*-CH_3–C_6H_4	119
32	*m*-CH_3–C_6H_4	120
33	*p*-CH_3–C_6H_4	123
34	2,4-$(CH_3)_2$–C_6H_3	120
35		7.37
36	$-CH_2-C_6H_5$	43.5
37	*m*-NO_2–C_6H_4	46
38	*P*-NO_2–C_6H_4	25
39	*P*-Br–C_6H_4	74.6
40	*p*-OCH_3–C_6H_4	26.7
41	*p*-OCH_2CH_3–C_6H_4	162
L-NNA		14.74

tency than at the 2-, or 4-position. Disubstitution of chloro group at the 2-, 4-position of the aromatic ring showed better potency than at the 2-, 5-position or 3-, 5-position. From the bioactivities of compounds **31**–**34**, mono-substitution of methyl group at the 2-, 3-, or 4-position and disubstitution of methyl group at the 2-, 4-position of the aromatic ring lead to the poor potency. Introducing strong electron-withdrawing group, nitro, showed moderate potency, for example compounds **37** and **38**. Other mono-substitution at the 4-position of aromatic ring such as bromine, methoxy, and ethoxy also showed low potency compared to the compound **17** (Fig. 2).



17

Figure 2. The structure of compound **17**.

3.2. Molecular modeling study

According to the 3D structure of murine iNOS (PDB ID 1r35),[9] FlexX docking was used for modeling the target compounds with the enzyme. Compound **17**, which showed best affinity in our target compounds, was selected for molecular docking utilizing Sybyl 7.0. Figure 3 (left and right) shows that phenyl group in compound **17** is in the bottom of the **C1** hydrophobic pocket of the enzyme and surrounded by hydrophobic amino acids, MET114, TRP457, and TYR485. There may be a H-bond between the heme propionate group and the carboxamido-group of compound **17**. There may be hydrogen bond interaction between the GLY345, GLU371, ASP376, GLN257, PRO344 and compound **17**.

4. Conclusion

We designed and synthesized a series of pyrrolidine derivatives as iNOS inhibitors and developed a convenient synthetic method to prepare target compounds. Several compounds were shown to possess potent iNOS activities. Among the inhibitors the best one was compound **17** (2.36 μM).

5. Experimental

5.1. iNOS inhibition assay (in vitro)

Inhibitory effects of test samples on the NO production in LPS-activated mouse macrophages were evaluated. Briefly, female Kunming mice, weighing 18–22 g, were treated with an intraperitoneal injection of 3% starch (1 mL), respectively. After 2 d, mice were put to death. Peritoneal exudate cells were collected from the peritoneal cavities of mice and the cells (10^6 cells/well) were suspended in 1 mL of Dulbecco's MEM (DMEM) supplemented with 5% fetal calf serum (FCS), penicillin (100 U/mL), and streptomycin (100 mg/mL), and precultured in 24-well microplates at 37 °C in 5% CO_2 in air for 4 h. Nonadherent cells were removed by washing the cells with D-hanks, and the adherent cells (more than 95% macrophages as determined by Giemsa staining) were cultured in fresh medium containing 10 μg/mL lipopolysaccharide (LPS) and various concentrations of test compound for 20 h. *L*-NNA was a specific inhibitor of NOS activity as a positive control. Nitrite and nitrate, the oxidized forms of nitric oxide that accumulated in the culture medium and plasma, were determined by the use of nitrite/nitrate colorimetric assay kit. Basically, the nitrate in the sample was reduced to nitrite with a nitrate reductase contained in the assay kit; nitrite levels were then determined spectrophotometrically as the total NO concentration. Inhibition (%) was calculated by the following formula and IC_{50} was determined graphically.

$$\text{Inhibition } (\%) = \frac{A-B}{A-C} \times 100$$

$A - C$: NO_2^- concentration

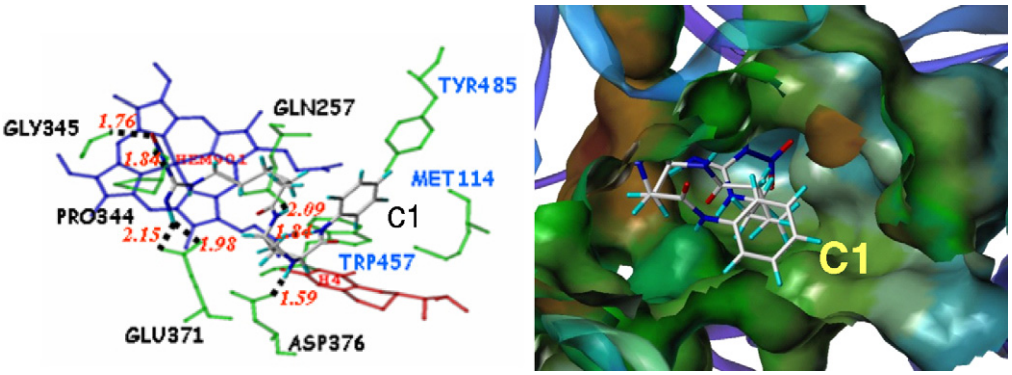


Figure 3. The FlexX docking of compound **17** with murine iNOS(PDB ID 1r35). Left: compound **17** in the active site of murine iNOS(PDB ID 1r35). The dash lines and numbers show the potential hydrogen bonds and bond length. The blue is heme; the red is tetrahydrobiopterin; the green is amino acid residues GLY 345, PRO344, GLU371, ASP376, GLN257, MET114, TRP457, and TYR485. The distances of the hydrogen bonds are shown in angstroms (Å). Right: phenyl group in compound **17** is in the bottom of the **C1** hydrophobic pocket of the murine iNOS (PDB ID 1r35).

[*A*: LPS (+), sample (−); *B*: LPS (+), sample (+); *C*: LPS (−), sample (−)].

5.2. Chemistry: general procedures

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. All the solvents except DMF have been distilled before use. All reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (60GF-254) and visualized with UV light or iodine vapor. Column chromatography was performed on silica gel (200–300 mesh). ESI-MS were determined on an API 4000 spectrometer. IR were recorded on a Nicolet Nexus 470 FT spectrometer. Melting points were determined on a electrothermal melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained on a brucker 600 at 600 MHz. The chemical shifts are expressed in δ values (parts per million) relative to tetramethylsilane (TMS) as internal standard. Significant ^{1}H NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet) number of protons.

5.2.1. (2*S*,4*R*)-Methyl 4-hydroxypyrrolidine-2-carboxylate hydrochloride[10] (7). The title compound was prepared as described by Jordis in (1*S*,4*S*)-2-thia-5-azabicyclo [2.2.1] heptane.

5.2.2. (2*S*,4*R*)-1-*tert*-Butyl 2-methyl 4-hydroxypyrrolidine-1,2-dicarboxylate (8). The title compound was prepared according to the synthesis of (2*R*,4*R*)-1-*tert*-butoxycarbonyl)-2-carbomethoxy-4-hydroxypyrrolidine described by Terry, R.; Daniel, T., W.; Chu, sabella, et al.[11] yield 92%. Mp: 91–94 °C; ^{1}H NMR (DMSO-d_6) δ 1.45 (s, 9H), 2.09 (dd, 1H, J = 6.3, 14.1 Hz), 2.34 (m, 1H), 3.5–3.74 (m, 3H), 3.79 (s, 3H), 4.34 (m, 2H); ESI-MS: *m*/*z* $[M+H]^+$246.

5.2.3. (2*S*,4*R*)-1-*tert*-Butyl 2-methyl 4-(methylsulfonyloxy)pyrrolidine-1,2-dicarboxylate (9). Under nitrogen atmosphere, in a 250 mL round-bottomed flask equipped with a magnetic stirbar and a rubber septum were placed compound **8** (12.67 g, 51.76 mmol) and CH_2Cl_2 (50 mL). To this stirring solution at 0 °C was added Et_3N (31.78 mL, 227.8 mmol) followed by methylsulfonyl chloride (8.98 mL, 113.89 mmol). The reaction mixture was stirred for 24 h (which time the ice bath expired), then diluted with CH_2Cl_2 (150 mL) and washed with saturated $NaHCO_3$, 10% citric acid, and brine in turn. The CH_2Cl_2 solution was dried over Na_2SO_4 and concentrated with a rotary evaporator to afford 16.7 g of orange oil, which solidified upon standing, yield 92%.

5.2.4. (2*S*,4*S*)-1-*tert*-Butyl 2-methyl 4-azidopyrrolidine-1,2-dicarboxylate (10). Under nitrogen atmosphere, compound **9** (16 g, 49.59 mmol) was taken in anhydrous DMF (30 mL) in the presence of ground NaN_3 (6.45 g, 99.13 mmol). The resulting mixture was heated to 60 °C for 24 h and then partitioned between water and EtOAc. The layers were separated and the organic phase was washed with water and brine in turn, dried over Na_2SO_4, filtered, and concentrated with a rotary evaporator to afford 11.65 g of orange oil, yield 87%.

5.2.5. (2*S*,4*S*)-1-*tert*-Butyl 2-methyl 4-aminopyrrolidine-1,2-dicarboxylate (11). In hydrogen atmosphere, compound **10** (14.34 g, 53.06 mmol) was dissolved in methanol and 5% $Pd/CaCO_3$ (2.4 g) was added. The resulting solution was stirred for 24 h under 760 mm Hg pressure. The resulting mixture was filtered and the filtrate was evaporated to give 9.3 g of pale yellow oil, yield 70%.

5.2.6. 6-Guanidinohexanoic acid (13). The title compound was prepared according to the synthesis of 7-guanidinoheptanoic acid described by Yoshihisa, U., Makoto, M., Hiroyuki, K., et al.,[12] yield 89%. Mp: >300 °C.

5.2.7. 6-(3-Nitroguanidino)hexanoic acid[13] (14). The title compound was prepared as described by Sadao Hashimoto et al. yield 78.2%. Mp: 156.5–158 °C. ^{1}H NMR (DMSO-d_6) δ 1.25–1.32 (m, 2H), 1.47–1.55 (m, 4H), 2.19–2.33 (t, J = 5.48 Hz, 2H), 3.11–3.16 (q, J = 4.84 Hz, 2H), 7.89–7.99 (br s, 2H), 8.52 (br s, 1 H), 11.98 (br s, 1H).

5.2.8. (2*S*,4*S*)-1-*tert*-Butyl 2-methyl 4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-1,2-dicarboxylate (15). To a stirred solution of compound **14** (13.58 g, 62.25 mmol) in THF (60 mL) were added at −5 °C DCC (14.12 g, 68.45 mmol) and HOBT (8.42 g, 68.45 mmol). The mixture was allowed to stand for 0.5 h at −5 °C. Then the compound **11** (19 g, 77.78 mmol) in solution in THF (40 mL) was added dropwise at 0 °C. After the addition was complete, the mixture was allowed to warm gradually to room temperature and left to stand one night. The solvent was concentrated under reduced pressure. EtOAc (100 mL) was added and the insoluble dicyclohexyl urea was eliminated by filtration. The filtrate was washed with brine, 10% citric acid, saturated $NaHCO_3$, and brine in turn. The EtOAc solution was dried over Na_2SO_4 and concentrated with a rotary evaporator under reduced pressure to afford 28.7 g of orange oil. The obtained residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 40:1) to afford compound **15** 23.24 g, yield 81%. Mp: 105–108 °C; IR (KBr, cm^{-1}) υ_{NH} 3321, υ_{CH} 2937, 2970, $\upsilon_{C=O}$ 1746, 1650, υ_{NH} 1540, υ_{CH} 1367, 1409, υ_{N-NO_2} 1261. ^1H NMR (DMSO-d_6) δ 1.32 (s, 9H), 1.46–1.49 (m, 6H), 1.74–1.79 (m, 1H), 2.02–2.06 (t, J = 5.41 Hz, 2H), 2.42–2.47 (m, 1H), 3.05–3.07 (t, J = 6.74 Hz, 1H), 3.10–3.15 (q, J = 4.85 Hz, 2H), 3.62–3.63 (m, 1H), 3.66 (s, 3H), 4.15–4.21 (m, 2H), 7.83 (br s, 2H), 7.91–7.94 (d, J = 4.55 Hz, 1H), 8.52 (br s, 1H). ESI-MS: m/z $[M+H]^+$445.7.

5.2.9. (2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxylic acid (16). To a stirred solution of compound **15** (20.71 g, 46.59 mmol) in DME (60 mL) was added 1 N NaOH (54.6 mL). The reaction mixture was stirred for 3 h. The pH of reaction mixture was adjusted to 5–6 with 1 N HCl. After removal of solvents, a residue was partitioned between EtOAc and 10% citric acid. The aqueous phase was extracted with EtOAc twice. The combined organic phase was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated with a rotary evaporator to give 20.05 g of crude oil. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 40:1) to afford compound **11** 18.05 g, yield 90%. Mp: 102–105 °C; IR (KBr, cm^{-1}) $\upsilon_{OH,NH}$ 3314, υ_{CH} 2937, $\upsilon_{C=O}$ 1731, 1653, υ_{NH} 1596, υ_{CH} 1430, υ_{N-NO_2} 1275. ^1H NMR (DMSO-d_6) δ 1.21–1.34 (s, 9H), 1.46–1.49 (m, 6H), 1.71–1.75 (m, 1H), 2.02–2.06 (t, J = 5.41 Hz, 2H), 2.42–2.47 (m, 1H), 3.05–3.07 (t, J = 6.74 Hz, 1H), 3.10–3.15 (q, J = 4.85 Hz, 2H), 3.62–3.63 (m, 1H), 4.15–4.21 (m, 2H). ESI-MS: m/z $[M+H]^+$431.7.

5.2.10. (2*S*,4*S*)-4-(6-(3-Nitroguanidino)hexanamido)-*N*-phenylpyrrolidine-2-carboxamide hydrochloride (17). To a stirred solution of compound **16** (5 g, 11.61 mmol) and *N*-methylmorpholine (1.40 mL, 12.75 mmol) in THF (30 mL) was added isobutyl chloroformate (1.66 mL, 12.75 mmol) at −15 °C. The mixture was stirred for 30 min at the same temperature. A solution of aniline (1.29 g, 13.93 mmol) in THF (20 mL) was added dropwise to the reaction mixture. The stirring was continued for 24 h at −5 °C, and the solvent was evaporated in vacuo. The residue was dissolved in EtOAc and washed with 5% $NaHCO_3$, 10% citric acid, and brine in turn. The EtOAc solution was dried over Na_2SO_4 and concentrated with a rotary evaporator to give 5.2 g of crude oil. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH 40:1) to afford white solid 4.22 g. The solid (1.15 g, 2.27 mmol) was dissloved in anhydrous EtOAc and then added dropwise 2 N HCl/EtOAc (10 mL) at 0 °C. The stirring was continued for 12 h. After removal of solvent, the obtained residue was purified by flash column chromatography (CH_2Cl_2:MeOH 6/1, then CH_2Cl_2:MeOH 3/1) to give 0.64 g of compound **17** as a white solid, yield 69%. Mp: 115–118 °C; IR (KBr, cm^{-1}) υ_{NH} 3240, υ_{Ar-H} 3060, υ_{CH} 2936, υ_{CH} 2863, $\upsilon_{C=O}$ 1689, 1631, $\upsilon_{Ar,C-C}$ 1596, υ_{NH} 1552, υ_{CH} 1448, υ_{N-NO_2} 1262. ^1H NMR (DMSO-d_6) δ 1.23–1.25 (m, 2H), 1.45–1.50 (m, 4H), 1.87–1.95 (m, 1H), 2.06–2.09 (m, 2H), 2.73–2.75 (m, 1H), 3.11–3.16 (br s, 3H), 3.45–3.50 (m, 1H), 4.34–4.38 (m, 1H), 4.42–4.44 (m, 1H), 7.11–7.13 (t, J = 7.38 Hz, 1H), 7.34–7.37 (t, J = 7.92 Hz, 2H), 7.62–7.63 (d, J = 7.67 Hz, 2H), 7.95 (br s, 2H), 8.12–8.13 (d, J = 6.44 Hz, 1H), 8.53 (br s, 1H), 8.86 (br s, 1H), 9.93 (br s, 1H), 10.75 (s, 1H). ESI-MS: m/z $[M+H]^+$ 406.8.

Compounds **18**–**41** were prepared as described for compound **17**.

5.2.11. (2*S*,4*S*)-*N*-(2-Fluorophenyl)-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (18). White solid, yield: 73%. Mp: 162–164 °C; IR (KBr, cm^{-1}) υ_{NH} 3295, υ_{Ar-H} 3052, υ_{CH} 2935, υ_{CH} 2855, $\upsilon_{C=O}$ 1696, 1646, $\upsilon_{Ar,C-C}$ 1588, υ_{NH} 1545, υ_{CH} 1458, υ_{N-NO_2} 1264. ^1H NMR (DMSO-d_6) δ 1.21–1.28 (m, 2H), 1.46–1.53 (m, 4H), 1.87–1.95 (m, 1H), 2.06–2.09 (m, 2H), 2.73–2.80 (m, 1H), 3.09–3.17 (m, 3H), 3.45–3.50 (m, 1H), 4.32–4.39 (m, 1H), 4.51–4.55 (t, J = 6.42 Hz, 1H), 7.20–7.33 (m, 3H), 7.79–7.88 (m, 1H), 7.95 (br s, 2H), 8.22–8.24 (d, J = 4.56 Hz, 1H), 8.50 (br s, 1H), 8.90 (br s, 1H), 10.31 (br s, 1H), 10.55 (br s, 1H). ESI-MS: m/z $[M+H]^+$ 424.6.

5.2.12. (2*S*,4*S*)-*N*-(4-Fluorophenyl)-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (19). White solid, yield: 67%. Mp: 145–146 °C; IR (KBr, cm^{-1}) υ_{NH} 3291, υ_{Ar-H} 3059, υ_{CH} 2938, υ_{CH} 2861, $\upsilon_{C=O}$ 1689, 1636, $\upsilon_{Ar,C-C}$ 1508, υ_{NH} 1541, υ_{CH} 1413, υ_{N-NO_2} 1266. ^1H NMR (DMSO-d_6) δ 1.15–1.28 (m, 2H), 1.45–1.52 (m, 4H), 1.85–1.92 (m, 1H), 2.04–2.08 (t, J= 5.52 Hz, 2H), 2.72–2.79 (m, 1H), 3.11–3.16 (m, 3H), 3.56 (m, 1H), 4.33–4.43 (m, 2H), 7.18–7.22 (t, J = 6.66 Hz, 2H), 7.65–7.68 (m, 2H), 7.91 (br s, 2H), 8.16–8.18 (d, J = 4.77 Hz, 1H), 8.50 (br s, 1H), 8.88 (br s, 1H), 10.05 (br s, 1H), 10.96 (br s, 1H). ESI-MS: m/z $[M+H]^+$ 424.5.

5.2.13. (2*S*,4*S*)-*N*-(2,4-Difluorophenyl)-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (20). White solid, yield: 74%. Mp: 125–128 °C; IR (KBr, cm^{-1}) υ_{NH} 3291, υ_{Ar-H} 3058, υ_{CH} 2929, υ_{CH} 2858, $\upsilon_{C=O}$ 1696, 1638, $\upsilon_{Ar,C-C}$ 1511, υ_{NH} 1541, υ_{CH} 1432, υ_{N-NO_2} 1262. ^1H NMR (DMSO-d_6) δ 1.21–1.26 (m, 2H), 1.46–1.51 (m, 4H), 1.86–1.91 (m, 1H), 2.05–

2.08 (t, J = 7.43 Hz, 2H), 2.72–2.77 (m, 1H), 3.08–3.16 (m, 3H), 3.56 (m, 1H) 4.32–4.38 (m, 1H), 4.45 (br s, 1H), 7.12–7.16 (m, 1H), 7.38–7.42 (m, 1H), 7.76–7.78 (m, 1H), 7.85–7.87 (d, J = 6.76 Hz, 1H), 7.93 (br s, 1H), 8.21–8.23 (d, J = 6.18 Hz, 1H), 8.53 (br s, 1H), 8.91 (br s, 1H), 10.09 (br s, 1H), 10.57 (s, 1H). ESI-MS: m/z $[M+H]^+$ 442.4.

5.2.14. (2*S*,4*S*)-*N*-(3,4-Difluorophenyl)-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (21). White solid, yield: 79%. Mp: 159–160 °C; IR (KBr, cm^{-1}) v_{NH} 3295, v_{Ar-H} 3065, v_{CH} 2942, $v_{C=O}$ 1693, 1634, v_{NH} 1541, $v_{Ar,C-C}$ 1517, v_{CH} 1423, v_{N-NO_2} 1266. ^{1}H NMR (DMSO-d_6) δ 1.20–1.25 (m, 2H), 1.45–1.50 (m, 4H), 1.83–1.88 (m, 1H), 2.04–2.08 (t, J = 7.40 Hz, 2H), 2.69–2.73 (m, 1H), 3.03–3.06 (m, 1H), 3.11 (br s, 2H), 3.43–3.46 (m, 1H), 4.31–4.38 (m, 2H), 7.39–7.41 (m, 1H), 7.43–7.48 (m, 1H), 7.79–7.83 (m, 1H), 7.93 (br s, 2H), 8.15–8.16 (d, J = 6.36 Hz, 1H), 8.51 (br s, 1H), 8.99 (br s, 1H), 11.11 (s, 1H). ESI-MS: m/z $[M+H]^+$ 442.8.

5.2.15. (2*S*,4*S*)-*N*-(3,5-Difluorophenyl)-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (22). White solid, yield: 75%. Mp: 140–142 °C; IR (KBr, cm^{-1}) v_{NH} 3294, v_{Ar-H} 3088, v_{CH} 2940, v_{CH} 2858, $v_{C=O}$ 1697, 1623, v_{NH} 1565, v_{CH} 1479, 1440, v_{N-NO_2}1265. ^{1}H NMR (DMSO-d_6) δ 1.25–1.26 (m, 2H), 1.47–1.51 (m, 4H), 1.88–1.93 (m, 1H), 2.06–2.09 (t, J = 5.34 Hz, 2H), 2.79–2.86 (m, 1H), 3.14 (br s, 3H), 3.49–3.57 (br s, 1H), 4.34–4.39 (m, 1H), 4.50 (br s, 1H), 6.96–7.00 (t, J = 6.84 Hz, 1H), 7.43–7.45 (d, J = 5.94 Hz, 2H), 7.96 (br s, 2H), 8.25–8.26 (d, J = 4.26 Hz, 1H), 8.51 (br s, 1H), 8.97 (br s, 1H), 10.21 (br s, 1H), 11.68 (s, 1H). ESI-MS: m/z $[M-H]^+$ 440.8.

5.2.16. (2*S*,4*S*)-4-(6-(3-Nitroguanidino)hexanamido)-*N*-(2,3,4-trifluorophenyl)pyrrolidine-2-carboxamide hydrochloride (23). White solid, yield: 65%. Mp: 132–134 °C; IR (KBr, cm^{-1}) v_{NH} 3296, v_{CH} 2941, $v_{CH}v_{C=O}$ 1694, 1639, v_{NH} 1543, $v_{Ar,C-C}$ 1513, v_{CH} 1482, v_{N-NO_2} 1266. ^{1}H NMR (DMSO-d_6) δ 1.20–1.26 (m, 2H), 1.45–1.50 (m, 4H), 1.88–1.91 (m, 1H), 2.04–2.08 (t, J = 7.41 Hz, 2H), 2.67–2.71 (m, 1H), 2.98–3.01 (t, J = 7.41 Hz, 1H), 3.14 (br s, 2H), 3.47–3.48 (m, 1H), 4.15–4.16 (br s, 1H), 4.29–4.32 (m, 1H), 4.39–4.41 (t, J = 8.50 Hz, 1H), 7.34–7.38 (m, 1H), 7.59–7.62 (m, 1H), 7.96 (br s, 1H), 8.17–8.18 (d, J = 6.29 Hz, 2H), 8.53 (br s, 1H), 8.97 (br s, 1H), 10.74 (s, 1H). ESI-MS: m/z $[M+H]^+$ 460.7.

5.2.17. (2*S*,4*S*)-*N*-(2-Chlorophenyl)-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (24). White solid, yield: 79%. Mp: 132–134 °C; IR (KBr, cm^{-1}) v_{NH} 3291, v_{Ar-H} 3052, v_{CH} 2936, v_{CH} 2861, $v_{C=O}$ 1685, 1638, $v_{Ar,C-C}$ 1593, v_{NH} 1538, v_{CH} 1442, v_{N-NO_2} 1267. ^{1}H NMR (DMSO-d_6) δ 1.22–1.26 (m, 2H), 1.46–1.51 (m, 4H), 1.88–1.93 (m, 1H), 2.05–2.08 (t, J = 7.48 Hz, 2H), 2.73–2.76 (m, 1H), 3.03–3.06 (t, J = 9.19 Hz, 1H), 3.11 (br s, 2H), 3.46–3.48 (m, 1H), 4.32–4.38 (m, 1H), 4.47–4.48 (t, J = 8.00 Hz, 1H), 7.26–7.29 (dt, J = 1.52, 7.71 Hz, 1H), 7.37–7.40 (dt, J = 1.33, 7.72 Hz, 1H), 7.54–7.55 (dd, J = 1.35, 8.03 Hz, 1H), 7.65–7.66 (d, J = 7.48 Hz, 1H), 7.92 (br s, 2H), 8.17–8.18 (d, J = 6.18 Hz, 1H), 8.53 (br s, 1H), 8.91 (br s, 1H), 9.89 (br s, 1H), 10.36 (s, 1H). ESI-MS: m/z $[M+H]^+$ 440.7.

5.2.18. (2*S*,4*S*)-*N*-(3-Chlorophenyl)-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (25). White solid, yield: 65%. Mp: 108–110 °C; IR (KBr, cm^{-1}) v_{NH} 3240, v_{Ar-H} 3059, v_{CH} 2934, v_{CH} 2858, $v_{C=O}$ 1692, 1634, $v_{Ar,C-C}$ 1596, v_{NH} 1544, v_{CH} 1430, v_{N-NO_2} 1280. ^{1}H NMR (DMSO-d_6) δ 1.20–1.25 (m, 2H), 1.45–1.50 (m, 4H), 1.87–1.92 (m, 1H), 2.04–2.08 (t, J = 7.42 Hz, 2H), 2.73–2.78 (m, 1H), 3.11 (m, 3H), 3.46–3.49 (m, 1H), 4.34–4.35 (m, 1H), 4.42–4.46 (t, J = 6.39 Hz, 1H), 7.17–7.20 (d, J = 5.91 Hz, 1H), 7.37–7.41 (t, J = 6.06 Hz, 1H), 7.53–7.55 (d, J = 6.81 Hz, 1H), 7.82–7.83 (t, J = 1.96 Hz, 1H), 7.92 (br s, 2H), 8.18–8.20 (d, J = 4.71 Hz, 1H), 8.5 (br s, 1H), 8.90 (br s, 1H), 10.06 (br s, 1H), 11.17 (s, 1H). ESI-MS: m/z $[M+H]^+$ 440.7.

5.2.19. (2*S*,4*S*)-*N*-(4-Chlorophenyl)-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (26). White solid, yield: 63%. Mp: 116–118 °C; IR (KBr, cm^{-1}) v_{NH} 3242, v_{Ar-H} 3056, v_{CH} 2939, v_{CH} 2858, $v_{C=O}$ 1692, 1631, $v_{Ar,C-C}$ 1492, v_{NH} 1547, v_{CH} 1397, v_{N-NO_2} 1287. ^{1}H NMR (DMSO-d_6) δ 1.21–1.25 (m, 2H), 1.45–1.52 (m, 4H), 1.85–1.88 (m, 1H), 2.05–2.08 (t, J = 7.40 Hz, 2H), 2.75–2.82 (m, 1H), 3.12–3.17 (br s, 3H), 3.47–3.48 (m, 1H), 4.32–4.36 (m, 1H), 4.42–4.45 (t, J = 4.40 Hz, 1H), 7.41–7.43 (d, J = 8.83 Hz, 2H), 7.69–7.71 (d, J = 8.87 Hz, 2H), 7.95 (br s, 2H), 8.20–8.21 (d, J = 6.02 Hz, 1H), 8.52 (br s, 1H), 8.89 (br s, 1H), 10.08 (br s, 1H), 11.13 (s, 1H). ESI-MS: m/z $[M+H]^+$ 440.7.

5.2.20. (2*S*,4*S*)-*N*-(2,4-Dichlorophenyl)-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (27). White solid, yield: 73%. Mp: 147–149 °C; IR (KBr, cm^{-1}) v_{NH} 3226-3382, v_{CH} 2937, $v_{C=O}$ 1696, 1635, $v_{Ar,C-C}$ 1589, v_{NH} 1532, v_{CH} 1383, v_{N-NO_2} 1287. ^{1}H NMR (DMSO-d_6) δ 1.23–1.26 (m, 2H), 1.46–1.51 (m, 4H), 1.90–1.93 (m, 1H), 2.05–2.08 (t, J = 7.42 Hz, 2H), 2.75–2.78 (m, 1H), 3.06–3.31 (m, 3H), 3.47–3.48 (m, 1H), 4.35–4.38 (m, 1H), 4.51–4.54 (m, 1H), 7.49–7.50 (dd, J = 2.36, 8.67 Hz, 1H), 7.66–7.68 (d, J = 8.68 Hz, 1H) 7.74–7.75 (d, J = 2.35 Hz, 1H), 7.89 (br s, 2H), 8.19–8.20 (d, J = 6.35 Hz, 1H), 8.51 (br s, 1H), 8.92 (br s, 1H), 10.0 (br s, 1H), 10.45 (s, 1H). ESI-MS: m/z $[M+H]^+$ 474.5.

5.2.21. (2*S*,4*S*)-*N*-(2,5-Dichlorophenyl)-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (28). White solid, yield: 71%. Mp: 124–126 °C; IR (KBr, cm^{-1}) v_{NH} 3296–3382, v_{Ar-H} 3052, v_{CH} 2936, $v_{C=O}$ 1691, 1639, $v_{Ar,C-C}$ 1588, v_{NH} 1531, v_{CH} 1404, v_{N-NO_2} 1265. ^{1}H NMR (DMSO-d_6) δ 1.22–1.26 (m, 2H), 1.46–1.50 (m, 4H), 1.90–1.93 (m, 1H), 2.05–2.08 (m, 2H), 2.75–2.78 (m, 1H), 3.07–3.10 (m, 3H), 3.45–3.48 (m, 1H), 4.32–4.34 (m, 1H), 4.50 (m, 1H), 7.33–7.35 (dd, J = 2.38, 8.71 Hz, 1H), 7.58–7.59 (d, J = 8.61 Hz, 1H), 7.84 (s, H), 7.90 (br s, 2H), 8.11–8.12 (d, J = 5.49 Hz,

1H), 8.61 (br s, 1H), 10.47 (s, 1H). ESI-MS: *m/z* $[M+H]^+$ 474.3.

5.2.22. (2*S*,4*S*)-*N*-(3,5-Dichlorophenyl)-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (29). White solid, yield: 65%. Mp: 149–152 °C; IR (KBr, cm^{-1}) υ_{NH} 3293, υ_{Ar-H} 3058, υ_{CH} 2936, υ_{CH} 2858, $\upsilon_{C=O}$ 1697, 1638, $\upsilon_{Ar,C-C}$ 1591, υ_{NH} 1545, υ_{CH} 1443, υ_{N-NO_2} 1270. ^{1}H NMR (DMSO-d_6) δ 1.20–1.25 (m, 2H), 1.44–1.49 (m, 4H), 1.88–1.93 (m, 1H), 2.04–2.08 (t, J = 7.46 Hz, 2H), 2.73–2.77 (m, 1H), 3.04–3.09 (m, 3H), 3.46–3.50 (m, 1H), 4.32–4.36 (m, 1H), 4.45–4.48 (t, J = 8.50 Hz, 1H), 7.37 (t, J = 1.88 Hz, 1H), 7.74 (d, J = 1.81 Hz, 2H), 7.86 (br s, 2H), 8.18–8.19 (d, J = 6.24 Hz, 1H), 8.53 (br s, 1H), 8.94 (br s, 1H), 9.94 (br s, 1H), 11.48 (br s, 1H). ESI-MS: *m/z* $[M+H]^+$ 474.5.

5.2.23. (2*S*,4*S*)-*N*-(3-Chloro-4-methylphenyl)-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (30). White solid, yield: 76%. Mp: 152–155 °C; IR (KBr, cm^{-1}) υ_{NH} 3297, υ_{Ar-H} 3052, υ_{CH} 2940, υ_{CH} 2855, $\upsilon_{C=O}$ 1689, 1638, $\upsilon_{Ar,C-C}$ 1605, υ_{NH} 1541, υ_{CH} 1497, 1377 υ_{N-NO_2} 1269. ^{1}H NMR (DMSO-d_6) δ 1.22–1.24 (m, 2H), 1.44–1.49 (m, 4H), 1.84–1.89 (m, 1H), 2.04–2.08 (t, J = 7.34 Hz, 2H), 2.28 (s, 3H), 2.67–2.72 (m, 1H), 3.04–3.10 (m, 3H), 3.44–3.45 (m, 1H), 4.36 (m, 2H), 7.32–7.34 (d, J = 8.28 Hz, 1H), 7.41–7.43 (d, J = 8.91 Hz, 1H), 7.8 (s, 1H), 7.92 (br s, 2H), 8.13–8.14 (d, J = 6.18 Hz, 1H), 8.51 (br s, 1H), 10.85 (s, 1H). ESI-MS: *m/z* $[M+H]^+$ 454.6.

5.2.24. (2*S*,4*S*)-4-(6-(3-Nitroguanidino)hexanamido)-*N*-*o*-tolylpyrrolidine-2-carboxamide hydrochloride (31). White solid, yield: 64%. Mp: 116–118 °C; IR (KBr, cm^{-1}) υ_{NH} 3291, υ_{Ar-H} 3052, υ_{CH} 2936, υ_{CH} 2858, $\upsilon_{C=O}$ 1693, 1638, $\upsilon_{Ar,C-C}$ 1596, υ_{NH} 1540, υ_{CH} 1390, υ_{N-NO_2} 1265. ^{1}H NMR (DMSO-d_6) δ 1.22–1.27 (m, 2H), 1.47–1.52 (m, 4H), 1.88–1.92 (m, 1H), 2.05–2.08 (t, J = 6.56 Hz, 2H), 2.22 (s, 3H), 2.76–2.81 (m, 1H), 3.07–3.16 (m, 3H), 3.48–3.50 (m, 1H), 4.35–4.40 (m, 1H), 4.49–4.52 (t, J = 8.70 Hz, 1H), 7.14–7.17 (dt, J = 1.10, 7.50 Hz, 1H), 7.20–7.22 (dt, J = 1.21, 7.60 Hz, 1H), 7.25–7.26 (d, J = 7.40 Hz, 1H), 7.36–7.37 (d, J = 7.24 Hz, 1H), 7.89 (br s, 2H), 8.23–8.24 (d, J = 6.36 Hz, 1H), 8.54 (br s, 1H), 8.86 (br s, 1H), 10.07 (br s, H), 10.17 (s, 1H). ESI-MS: *m/z* $[M+H]^+$ 420.6.

5.2.25. (2*S*,4*S*)-4-(6-(3-Nitroguanidino)hexanamido)-*N*-*m*-tolylpyrrolidine-2-carboxamide hydrochloride (32). White solid, yield: 71%. Mp: 158–160 °C; IR (KBr, cm^{-1}) υ_{NH} 3295, υ_{Ar-H} 3052, υ_{CH} 2937, υ_{CH} 2858, $\upsilon_{C=O}$ 1693, 1635, $\upsilon_{Ar,C-C}$ 1596, υ_{NH} 1541, υ_{CH} 1430, υ_{N-NO_2} 1275. ^{1}H NMR (DMSO-d_6) δ 1.20–1.27 (m, 2H), 1.45–1.52 (m, 4H), 1.86–1.94 (m, 1H), 2.05–2.08 (t, J = 5.54 Hz, 2H), 2.29 (s, 3H), 2.72–2.79 (m, 1H), 3.11 (br s, 3H), 3.45–3.50 (m, 1H), 4.34–4.38 (m, 1H), 4.42–4.46 (t, J = 6.39 Hz, 1H), 6.93–6.95 (d, J = 5.64 Hz, 1H), 7.21–7.25 (t, J = 6.09 Hz, 1H), 7.45–7.45 (d, J = 1.23 Hz, 2H), 7.93 (br s, 2H), 8.20–8.22 (d, J = 4.74 Hz, 1H), 8.51 (br s, 1H), 8.86 (br s, 1H), 10.16 (br s, 1H), 10.82 (s, 1H). ESI-MS: *m/z* $[M+H]^+$ 420.6.

5.2.26. (2*S*,4*S*)-4-(6-(3-Nitroguanidino)hexanamido)-*N*-*p*-tolylpyrrolidine-2-carboxamide hydrochloride (33). White solid, yield: 78%. Mp: 148–150 °C; IR (KBr, cm^{-1}) υ_{NH} 3422, υ_{CH} 2936, υ_{CH} 2858, $\upsilon_{C=O}$ 1693, 1635, $\upsilon_{Ar,C-C}$ 1513, υ_{NH} 1548, υ_{N-NO_2} 1295. ^{1}H NMR (DMSO-d_6) δ 1.21–1.25 (m, 2H), 1.44–1.52 (m, 4H), 1.83–1.91 (m, 1H), 2.05–2.08 (t, J = 7.41 Hz, 2H), 2.26 (s, 3H), 2.71–2.75 (m, 1H), 3.05–3.06 (m, 3H), 3.48–3.50 (m, 1H), 4.34–4.42 (m, 2H), 7.15–7.17 (d, J = 8.38 Hz, 2H), 7.50–7.55 (d, J = 8.42 Hz, 2H), 7.83 (br s, 2H), 8.20–8.21 (d, J = 6.44 Hz, 1H), 8.53 (br s, 1H), 8.86 (br s, 1H), 9.97 (br s, 1H), 10.74 (br s, 1H). ESI-MS: *m/z* $[M+H]^+$ 420.6.

5.2.27. (2*S*,4*S*)-*N*-(2,4-Dimethylphenyl)-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (34). White solid, yield: 77%. Mp: 125–127 °C; IR (KBr, cm^{-1}) υ_{NH} 3291, υ_{Ar-H} 3052, υ_{CH} 2938, υ_{CH} 2858, $\upsilon_{C=O}$ 1638, $\upsilon_{Ar,C-C}$ 1586, υ_{NH} 1539, υ_{CH} 1391, υ_{N-NO_2} 1270. ^{1}H NMR (DMSO-d_6) δ 1.21–1.26 (m, 2H), 1.46–1.51 (m, 4H), 1.86–1.91 (m, 1H), 2.05–2.08 (t, J = 7.71 Hz, 2H), 2.15 (s, 3H), 2.25 (s, 3H), 2.73–2.77 (m, 1H), 3.05–3.06 (m, 3H), 3.47 (m, 1H), 4.35–4.37 (t, J = 7.73 Hz, 1H), 4.44 (m, 1H), 7.00–7.01 (d, J = 8.14 Hz, 1H), 7.06 (s, 1H), 7.20–7.21 (d, J = 8.01 Hz, 1H), 7.82 (br s, 2H), 8.17–8.18 (d, J = 6.46 Hz, 1H), 8.53 (br s, 1H), 8.84 (br s, 1H), 9.78 (br s, 1H), 9.98 (s, 1H). ESI-MS: *m/z* $[M+H]^+$ 434.6.

5.2.28. (2*S*,4*S*)-*N*-Cyclohexyl-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (35). White solid, yield: 66%. Mp: 143–145 °C; IR (KBr, cm^{-1}) υ_{NH} 3276, υ_{Ar-H} 3065, υ_{CH} 2932, υ_{CH} 2855, $\upsilon_{C=O}$ 1652, $\upsilon_{Ar,C-C}$ 1596, υ_{NH} 1546, υ_{N-NO_2} 1269. ^{1}H NMR (DMSO-d_6) δ 1.16–1.29 (m, 7H), 1.45–1.53 (m, 5H), 1.65–1.77 (m, 5H), 2.05–2.08 (t, J = 5.54 Hz, 2H), 2.60–2.64 (m, 1H), 3.02–3.07 (t, J = 7.03 Hz, 1H), 3.13–3.17 (br s, 2H), 3.44 (m, 1H), 3.57–3.58 (m, 1H), 4.14–4.19 (t, J = 6.44 Hz, 1H), 4.28–4.33 (m, 1H), 7.96 (br s, 2H), 8.18–8.20 (d, J = 4.83 Hz, 1H), 8.51–8.53 (d, J = 5.76 Hz, 2H), 8.68 (br s, 1H), 10.13 (br s, 1H). ESI-MS: *m/z* $[M+H]^+$ 412.5.

5.2.29. (2*S*,4*S*)-*N*-Benzyl-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (36). White solid, yield: 63%. Mp: 118–120 °C; IR (KBr, cm^{-1}) υ_{NH} 3233, υ_{Ar-H} 3063, υ_{CH} 2934, υ_{CH} 2858, $\upsilon_{C=O}$ 1673, 1643, $\upsilon_{Ar,C-C}$ 1586, υ_{NH} 1541, υ_{CH} 1430, υ_{N-NO_2} 1265. ^{1}H NMR (DMSO-d_6) δ 1.22–1.26 (m, 2H), 1.45–1.50 (m, 4H), 1.74–1.77 (m, 1H), 2.05–2.08 (t, J = 7.42 Hz, 2H), 2.62–2.65 (m, 1H), 3.00–3.02 (m, 1H), 3.12–3.15 (br s, 2H), 3.45–3.57 (m, 1H), 4.24–4.25 (m, 1H), 4.31–4.38 (m, 3H), 7.26–7.29 (m, 3H), 7.32–7.35 (m, 2H), 7.96 (br s, 2H), 8.16–8.17 (d, J = 6.52 Hz, 1H), 8.52 (br s, 1H), 8.80 (br s, 1H), 9.10–9.12 (t, J = 5.77 Hz, 1H), 9.85 (br s, 1H). ESI-MS: *m/z* $[M+H]^+$ 420.7.

5.2.30. (2*S*,4*S*)-4-(6-(3-Nitroguanidino)hexanamido)-*N*-(3-nitrophenyl)pyrrolidine-2-carboxamide hydrochloride (37). Pale yellow oil. yield: 60%. IR (KBr, cm^{-1}) υ_{NH} 3215, υ_{Ar-H} 3048, υ_{CH} 2938, υ_{CH} 2858, $\upsilon_{C=O}$ 1697, 1639, $\upsilon_{Ar,C-C}$ 1596, 1511, υ_{NH} 1566, υ_{CH} 1343, υ_{N-NO_2}

1259. ^{1}H NMR (DMSO-d_6) δ 1.20–1.24 (m, 2H), 1.44–1.49 (m, 4H), 1.90–1.92 (m, 1H), 2.04–2.08 (t, J = 7.45 Hz, 2H), 2.70–2.75 (m, 1H), 3.04–3.10 (m, 3H), 3.44–3.47 (m, 1H), 4.31–4.33 (m, 1H), 4.39–4.42 (t, J = 8.32 Hz, 1H), 7.66–7.69 (t, J = 8.19 Hz, H), 7.89 (br s, 2H), 7.96–8.00 (m, 3H), 8.14–8.15 (d, J = 6.26 Hz, 1H), 8.52 (br s, 1H), 8.68 (s, 1H), 11.26 (s, 1H). ESI-MS: m/z $[M+H]^+$451.6.

5.2.31. (2*S*,4*S*)-4-(6-(3-Nitroguanidino)hexanamido)-*N*-(4-nitrophenyl)pyrrolidine-2-carboxamide hydrochloride (38). White solid, yield: 69%. Mp: 148–150 °C; IR (KBr, cm^{-1}) υ_{NH} 3382, υ_{Ar-H} 3052, υ_{CH} 2936, υ_{CH} 2861, $\upsilon_{C=O}$ 1693, 1635, $\upsilon_{Ar,C-C}$ 1596, υ_{NH} 1530, υ_{CH} 1351, υ_{N-NO_2} 1262. ^{1}H NMR (DMSO-d_6) δ 1.21–1.24 (m, 2H), 1.44–1.48 (m, 4H), 1.89–1.91 (m, 1H), 2.04–2.08 (t, J = 7.41 Hz, 2H), 2.77–2.79 (m, 1H), 3.09–3.15 (br s, 3H), 4.33–4.36 (m, 1H), 4.51 (m, 1H), 7.83 (br s, 2H), 7.90–7.92 (d, J = 9.22 Hz, 2H), 8.18–8.19 (d, J = 6.22 Hz, 1H), 8.26–8.28 (d, J = 9.24 Hz, 2H), 8.53 (br s, 1H), 8.97 (br s, 1H), 9.97 (br s, 1H), 11.59 (s, 1H). ESI-MS: m/z $[M+H]^+$ 451.6.

5.2.32. (2*S*,4*S*)-*N*-(4-Bromophenyl)-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (39). White solid, yield: 73%. Mp: 132–135 °C; IR (KBr, cm^{-1}) υ_{NH} 3246, υ_{Ar-H} 3053, υ_{CH} 2934, υ_{CH} 2858, $\upsilon_{C=O}$ 1691, 1634, υ_{NH} 1545, $\upsilon_{Ar,C-C}$ 1489, υ_{CH} 1394, υ_{N-NO_2} 1285. ^{1}H NMR (DMSO-d_6) δ 1.20–1.25 (m, 2H), 1.45–1.52 (m, 4H), 1.84–1.89 (m, 1H), 2.04–2.08 (t, J = 7.40 Hz, 2H), 2.73–2.77 (m, 1H), 3.07–3.12 (m, 3H), 3.43–3.49 (m, 1H), 4.32–4.38 (m, 1H), 4.42–4.47 (m, 1H), 7.53–7.55 (d, J = 6.76 Hz, 2H), 7.63–7.65 (d, J = 6.81 Hz, 2H), 7.94 (br s, 2H), 8.21–8.23 (d, J = 4.68 Hz, 1H), 8.51 (br s, 1H), 8.90 (br s, 1H), 10.19 (br s, 1H), 11.18 (s, 1H). ESI-MS: m/z $[M+H]^+$ 485.

5.2.33. (2*S*,4*S*)-*N*-(4-Methoxyphenyl)-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (40). White solid, yield: 67%. Mp: 160–162 °C; IR (KBr, cm^{-1}) υ_{NH} 3299-3382, υ_{Ar-H} 3052, υ_{CH} 2938, $\upsilon_{C=O}$ 1639, υ_{NH} 1552, $\upsilon_{Ar,C-C}$ 1512, υ_{CH} 1416, υ_{N-NO_2} 1244. ^{1}H NMR (DMSO-d_6) δ 1.22–1.25 (m, 2H), 1.46–1.50 (m, 4H), 1.88–1.90 (m, 1H), 2.05–2.07 (t, J = 7.43 Hz, 2H), 2.70–2.73 (m, 1H), 3.08–3.16 (m, 3H), 3.43–3.45 (m, 1H), 3.73 (s, 3H), 4.35–4.37 (m, 2H), 6.92–6.93 (d, J = 6.86 Hz, 2H), 7.52–7.53 (d, J = 6.88 Hz, 2H), 7.89 (br s, 2H), 8.12–8.13 (d, J = 6.42 Hz, 1H), 8.51 (br s, 1H), 8.81 (br s, 1H), 9.89 (br s, 1H), 10.59 (s, 1H). ESI-MS: m/z $[M+H]^+$ 436.5.

5.2.34. (2*S*,4*S*)-*N*-(4-Ethoxyphenyl)-4-(6-(3-nitroguanidino)hexanamido)pyrrolidine-2-carboxamide hydrochloride (41). White solid, yield: 75%. Mp: 154–156 °C; IR (KBr, cm^{-1}) υ_{NH} 3247, υ_{Ar-H} 3052, υ_{CH} 2934, υ_{CH} 2858, $\upsilon_{C=O}$ 1684, 1635, υ_{NH} 1550, $\upsilon_{Ar,C-C}$ 1511, υ_{CH} 1430, υ_{N-NO_2} 1243. ^{1}H NMR (DMSO-d_6) δ 1.17–1.20 (m, 2H), 1.29–1.31 (t, J = 6.96 Hz, 3H), 1.40–1.45 (m, 4H), 1.70–1.72 (m, 1H), 1.99–2.02 (t, J = 7.44 Hz, 2H), 2.42–2.44 (m, 1H), 2.74–2.75 (m, 1H), 3.09 (br s, 2H), 3.11–3.18 (m, 1H), 3.87–3.95 (m, 1H), 3.95–3.99 (q, J = 6.93 Hz, 2H), 4.13–4.14 (m, 1H), 6.85–6.88 (d, J = 9.01 Hz, 2H), 7.54–7.56 (d, J= 9.04 Hz, 2H), 7.89 (br s, 2H), 7.98–8.00 (d, J = 6.68 Hz, 1H), 8.51 (br s, 1H), 10.09 (s, 1H). ESI-MS: m/z $[M+H]^+$ 450.7.

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