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Design, synthesis, inhibitory activity, and SAR studies of pyrrolidine derivatives as neuraminidase inhibitors

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Abstract—A series of pyrrolidine derivatives were synthesized and evaluated for their ability to inhibit neuraminidase (NA) of influenza A virus (H3N2). All compounds were synthesized in good yields starting from commercially 4-hydroxy-L-proline using a suitable synthetic strategy. These compounds showed potent inhibitory activity against influenza A neuraminidase. Within this series, five compounds, **6e**, **9c**, **9e**, **9f**, and **10e**, have good potency (IC_{50} = 1.56–2.71 μM) which are compared to that the NA inhibitor Oseltamivir (IC_{50} = 1.06 μM), and could be used as lead compoundS in the future.
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1. Introduction

Influenza is an acute viral infection of the upper respiratory tract that can affect millions of people every year.[1] Vaccines against influenza virus are ineffective due to the rapid emergence of mutant viral antigens. The M2 protein ion channel blockers, for example amantadine and rimantadine, are only effective on type A influenza with undesirable side effects and rapidly generated resistant mutants.[2] Because effective and safe anti-influenza therapeutics are lacking, developing effective anti-influenza agents has become a high-priority and attractive area in drug discovery.

Hemagglutinin (HA) and neuraminidase (NA) are two glycoproteins in viral surface and essential for viral replication, infectivity, and the infective cycle of influenza. HA is known to mediate binding of viruses to target cells via terminal sialic acid (SA) residue in glycoconjugates.[3] In contrast to HA activity, NA catalyzes removal of terminal SA linked to glycoproteins and glycolipids. It has been suggested that NA is not only crucial in the release of virion progeny away from infected cells,[4] but also important in the movement of the virus through mucus of respiratory tract and reducing the propensity of the virus particles to aggregate. Despite the homological identity of NA in different strains being only about 30%, the catalytic site of neuraminidase in all influenza A and B viruses is completely conserved.[5] Therefore, NA has been regarded as an attractive target for antiviral drug development. Now, two NA inhibitors, Zanamivir and Tamiflu, have been confirmed as effective and safe for the treatment of influenza and approved by FDA.[6]

In 1999, Luo et al., proposed that the enzymatic active site contains four anti-parallel strands arranged in a propeller fashion.[7] Each monomeric subunit has an active site cavity lined with 10 conserved residues and four water molecules. The inhibitors bind to the active site with the carboxylic acid binding to the triad guanidine groups of the three arginine residues, Arg118, Arg371, and Arg292, which are located as a cluster on one side of the active site. Opposite to the guanidine triad, there is a hydrophobic pocket formed by side chains of Trp178, Ile 222, and part of Arg224. In the design of inhibitor Oseltamivir,[22] an amino group was used to replace the 4-OH of SA and interact with a negatively charged pocket formed by Glu119 and Glu227. Wang et al.[8] derived an 'airplane' model of the NA active site as illustrated in Figure 1 to summarize the basic structural requirements of a potent NA inhibitor.

Keywords: Neuraminidase inhibitor; SAR study; Pyrrolidine derivatives; Peptidomimetics.
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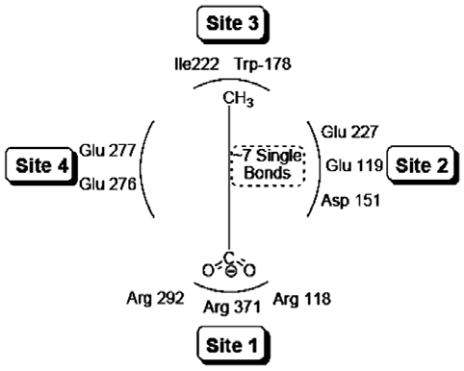
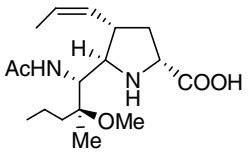


Figure 1. 'Airplane' model of NA active site (Ref. 8).

According to the studies on NA active site and SAR of published NA inhibitors, inhibition of the NA is mainly determined by the relative positions of the substituents (carboxylate, glycerol, acetamido, and hydroxyl) of the central ring. Currently, several pyrrolidine compounds have been found to possess potent NA inhibitory activities.[7,8] For example, A-315675[8] (**1**) is highly active in cell culture against a variety of strains of influenza A and B viruses. This urges us to develop new NA inhibitors based on pyrrolidine derivatives which contain different substituents (carboxylate, guanidino, acetamino, and alkyl) to interact with the four binding pockets of the NA active site.[9]



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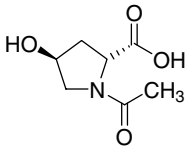
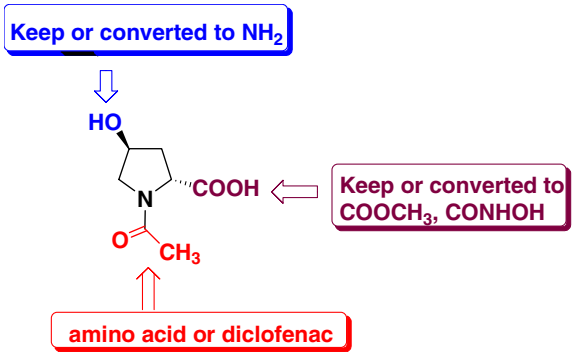


2. NAHYP

In our previous work, the 4-hydroxy-L-proline had been used to prepare a series of pyrrolidine derivatives as matrix metalloproteinase (MMP) and aminopeptidase-*N* (APN) inhibitors.[10,11] We first screened all the pyrrolidine derivatives in our compound library including not only the target compounds and intermediates we synthesized before, but also some commercially available compounds. The pharmacological result showed that NAHYP, one anti-inflammation compound (**2**, Oxaceprol), exhibited modest activity against influenza virus A (H_3N_2) neuraminidase (IC_{50} = 48.73 μM) and could be used as lead compound in future.

In order to improve the affinity of lead compounds, we optimized the structure of NAHYP with the following chemical modification: (i) *N*-acyl group in pyrrolidine ring was changed to other Boc-protected or free amino acid residues; (ii) 2-carboxylic acid can be kept or converted to other derivatives such as methyl ester or hydroxymate; (iii) keep hydroxy in 4-position or converted to free amino group. Despite the designed dipeptides serving as target compounds, a series of peptidomimetics have been designed by acylating pyrrolidine with diclofenac which is a known NSAIDs so as to enhance the stability of the target compounds.[12]



2. Chemistry

The synthesis of pyrrolidine derivatives possessing NA inhibitory activities is shown in Scheme 1. The starting materials, Boc-protected amino acids (**3**) and the 4-hydroxy-L-proline methyl ester hydrochloride (**4**), were prepared according to the literature.[10] The Boc-amino acids (**3**) were activated with DCC and HOBt and then coupled with compound **4** to yield **5a**–**10a**. The methy-lester **5a**–**10a** was then hydrolyzed with $NaOH/H_2O$ or treated with NH_2OK to obtain relative carboxylic acids **5b**–**10b** or hydroximic acids **5c**–**10c**.[13]

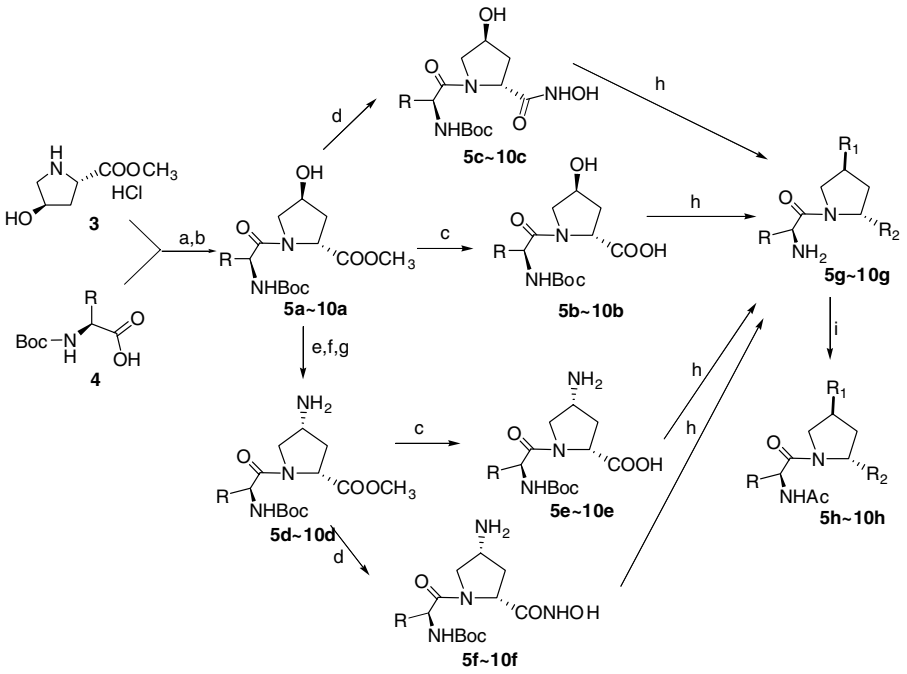
4-Amino-pyrrolidine derivatives were prepared from intermediate **5a**–**10a**. First, the hydroxyl group of **5a**–**10a** was convert to mesyl group by methanesulfonate and then reacted with sodium azide to generate configuration inverted azide. Amino derivatives **5d**–**10d** can be synthesized by hydrogenation using Pd–$CaCO_3$. The methyl ester **5d**–**10d** can be transformed to corresponding carboxylic acids **5e**–**10e** and hydroximic acids **5f**–**10f** as the methods mentioned before.

The Boc-protecting group can be easily removed with 3 N HCl in ethyl acetate to give hydrochloride salts of pyrrolidine derivatives **5g**–**10g**. Finally, the amino group was acetylated with acetic anhydride to yield **5h**–**10h**.

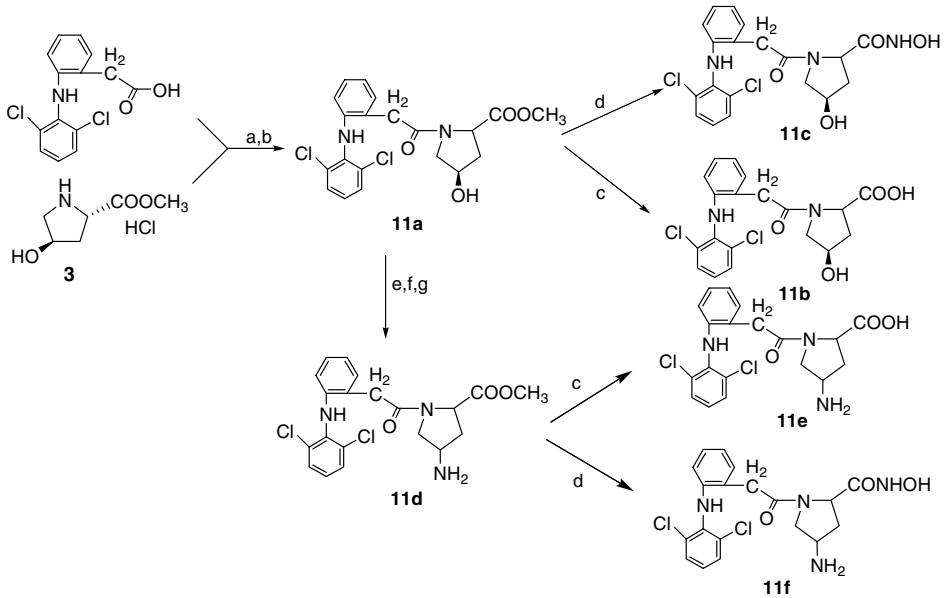
The synthesis of peptidomimetics that possess NA inhibitory activity is shown in Scheme 2. This scheme is similar to Scheme 1, the coupling of diclofenac with L-hydroxyproline using the strategy of synthesis of dipeptide.

3. Results and discussion

All the target compounds (47 compounds) were tested for their ability to inhibit neuraminidase. Preliminary result showed that 24 compounds displayed inhibitory activities with IC_{50} value from 1.56 to 90.79 μM (Table 1). Compound **10e** with NH_2 group and methionine as



Scheme 1. Reagents and conditions: (a) DCC, HOBt, THF, 0 °C; (b) compound **3**, NMM, THF; (c) MeOH, NaOH/H_2O, 25 °C; (d) NH_2OK, MeOH, 65 °C; (e) MsCl, Et_3N, DCM; (f) NaN_3, DMF, 65 °C; (g) 5% Pd–$CaCO_3$, H_2, MeOH; (h) HCl/EtOAC, EtOAc, 25 °C; (i) Ac_2O, Et_3N, DCM.

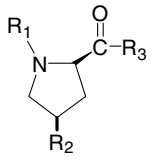


Scheme 2. Reagents and conditions: (a) DCC, HOBt, THF, 0 °C; (b) compound **3** NMM, THF; (c) MeOH, NaOH/H_2O, 25 °C; (d) NH_2OK, MeOH, 65 °C; (e) MsCl, Et_3N, DCM; (f) NaN_3, DMF, 65 °C; (g) 5% Pd–$CaCO_3$, H_2, MeOH.

hydrophobic side-chain showed the best inhibitory activity (IC_{50} = 1.56 μM). Three compounds containing isoleucine (**10c**, **10e**, and **10f**) exhibited good activities (2.10–2.71 μM). Compounds, **11c** and **11f**, had lower IC_{50} value (8.54 and 3.57 μM), and they possibly had anti-inflammatory activity. Generally, the compound with NH_2 and –COOH group showed better activities than that with OH and –CONHOH group.

In summary, our studies have discovered a new series of pyrrolidine derivatives that have potent NA inhibitory activity. The binding of compound **10e** in the active site of NA is shown in Figure 2, and we found that the $–CO_2H$ or –CONHOH group of the target compounds interacts with the positively charged site 1 of the NA active site (Fig. 1), the exocyclic OH or NH_2 group binds to the negatively charged site 2, and the Boc group

Table 1. The structure and in vitro inhibitory activities of compounds against NA



Compound	R_1	R_2	R_3	IC_{50}(μM)	$pIC_5^{act}0$	$pIC_5^{pre}0$	Res.
2	CH_3CO	OH	OH	48.73	3.55	3.64	−0.091
5b	Boc-Leu	OH	OH	11.51	4.47	4.92	−0.445
5c	Boc-Leu	OH	NHOH	3.87	4.96	4.91	0.049
5f	Boc-Leu	NH_2	NHOH	6.13	4.76	4.92	−0.162
6b	Boc-Phe	OH	OH	2.85	5.12	5.08	0.033
6c	Boc-Phe	OH	NHOH	4.27	4.96	5.08	−0.12
6e	Boc-Phe	NH_2	OH	2.40	5.19	5.15	0.037
6f	Boc-Phe	NH_2	NHOH	4.17	4.97	5.03	−0.060
7b	Boc-Val	OH	OH	4.63	4.85	4.94	−0.093
7c	Boc-Val	OH	NHOH	6.09	4.75	4.94	−0.188
7e	Boc-Val	NH_2	OH	4.29	4.88	5.03	−0.148
8a	Boc-Ala	OH	OMe	90.79	3.54	3.59	−0.048
8d	Boc-Ala	NH_2	OMe	83.69	3.57	3.62	−0.052
8e	Boc-Ala	NH_2	OH	15.82	4.27	4.26	0.014
8f	Boc-Ala	NH_2	NHOH	12.62	4.39	4.22	0.177
9c	Boc-Ile	OH	NHOH	2.15	5.22	5.12	0.096
9e	Boc-Ile	NH_2	OH	2.71	5.10	5.22	−0.122
9f	Boc-Ile	NH_2	NHOH	2.10	5.23	5.15	0.080
10e	Boc-Met	NH_2	OH	1.56	5.36	5.35	0.010
11b	DCA[a]	OH	OH	19.81	4.31	4.26	0.051
11c	DCA	OH	NHOH	8.54	4.70	4.69	0.001
11e	DCA	NH_2	OH	16.23	4.40	4.41	−0.015
11f	DCA	NH_2	NHOH	3.57	5.07	5.11	−0.040
5g	Leu	NH_2	OMe	48.01	3.72	4.22	−0.497
Oseltamivir				1.06	5.49		

[a] DCA is 2-(2-(2,6-dichlorophenylamino)phenyl)acetic acid.

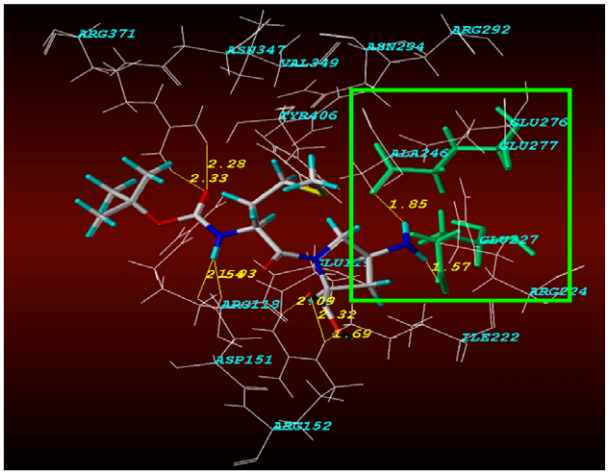


Figure 2. FlexX-docked result. Compound**10e** in the active site of neuraminidase (PDB ID:1nnc). The yellow lines and numbers show the potential hydrogen bonds and bond length.

interacts with the hydrophobic site 3 via one of the methyl groups. The hydrophobic side chain occupied the hydrophobic pocket of site 4. Compared to the other research, we reported a more convenient and economical method for the synthesis of pyrrolidine NA inhibitors. Compared to the other research, 4-hydroxy-L-proline we used appeared to be an ideal starting material because of its low cost and commercial abundance.

4. SAR studies

4.1. Dataset and molecular modeling

We used Sybyl7.0 program to carry out the SAR studies of these pyrrolidine derivatives. The CoMFA studies were carried out with the QSAR model of Sybyl. The test set consisted of **5b** and **7e**, the other 22 compounds composed of the training set. The IC_{50} values were converted into pIC_{50} according to the formula: $pIC_{50} = \log_{10}IC_{50}$.

Based on the docking results, the template molecule **2** was taken and the rest of the molecules were aligned to it using the pyrrolidine as scaffold by DATABASE ALIGNMENT method in the Sybyl. The aligned molecules are shown in Figure 3.

The steric and electrostatic CoMFA fields were calculated at each lattice intersection of a regularly spaced grid of 2.0 Å in all three dimensions within defined region. An sp^3 carbon atom with +1.00 charge was used as a probe atom. The steric and electrostatic fields were truncated at +30.00 kcal mol^{-1}, and the electrostatic fields

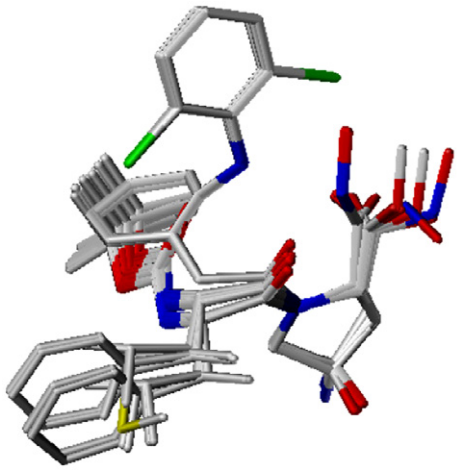


Figure 3. Superposition of 22 inhibitors for CoMFA construction.

were ignored at the lattice points with maximal steric interactions.

PLS method was used to linearly correlate the CoMFA fields to the inhibitory activity values. The cross-validation analysis was performed using the leave one out (LOO) method in which one compound is removed from the dataset and its activity is predicted using the model derived from the rest of the dataset. The cross-validated q^2 (0.852) that resulted in optimum number of components ($n = 5$) and lowest standard error of prediction were considered for further analysis. We have evaluated different filter value σ and at least selected σ as 2.00 kcal mol^{-1} to speed up the analysis and reduce noise.

4.2. Results and discussion

Only from the docked results we can obtain the conclusions: the order of increasing activity is R_2: –NHOH> –OH>–OMe, R_3: $–NH_2$>–OH, which we can also find from the actual results. We have found two potential hydrogen bonds (respectively to Glu277 and Glu227) when the R_3 is $–NH_2$ (Fig. 2), which was not existing when the R_3 was –OH. The QSAR model obtained from steric and electrostatic fields displayed good correlation with the LOO cross-validated q^2 of 0.852 at five optimum components and SEE of 0.096. For CoMFA, a five-component model was obtained with q^2, r^2, F, and SEE of 0.852, 0.978, 141.328, and 0.096, respectively. We also found in the CoMFA model that the contributions of steric and electrostatic fields are 0.541 and 0.459, respectively.

From Figure 4(b) we can find that the CoMFA model can predict **7e** well, but not very well **5b** in the test set and **11b** in the training set. That may have been caused by the sample size being much more lower when the actual $pIC_{50} < 4.7$ than it >4.7.

5. Conclusions

We have described the synthesis and properties of a series of pyrrolidine derivatives as influenza NA inhibitors. Several compounds were shown to possess potent influenza NA inhibitory activity, although in all cases, measured activity was lower than that of Oseltamivir. The most potent compound of the series is compound **10e** ($IC_{50} = 1.56$ μM), which, in addition to good enzyme inhibitory activity, displays potent anti-viral activity in vitro. We reported a more convenient and economical method for the synthesis of pyrrolidine NA inhibitors. Compared to the other research, 4-hydroxy-L-proline we used appeared to be an ideal starting material because of its low cost and commercial abundance. Accurate structural knowledge of the inhibitors bound to the active site made it clear that at least three regions of the active site of NA needed to be occupied to establish a consistent binding orientation, S1, S2, and S3, (Fig. 1) for our NAHYP-based pyrrolidine derivatives. Establishing a consistent binding mode was critical to predictive structure-based drug design and discovering potent compounds in the nanomolar range that would potentially be useful for antiviral therapy. The compounds we have got all showed potent NA inhibitory activity, and this finding could be used to design further influenza NA inhibitors.

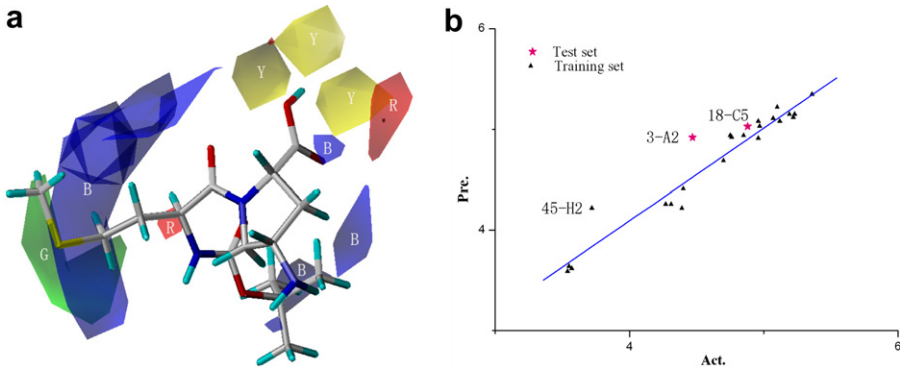


Figure 4. (a) The most active molecule F5 is shown in the background. Red (R) color represents the negative charge region, blue (B) is the positive charge region, green (G) is the more bulky region, and yellow (Y) is the less bulky region. (b) The predictability of the CoMFA model.

6. Experimental

6.1. Neuraminidase inhibition assay

The in vitro assay is based on the method reported by Guahua Du.[14,15] The strain A (Yuefang 72-243 A and Jifang 90-15 A) influenza viruses, which were donated by Chinese Centers for Disease Control, were used as source of NA. The NA was obtained by the method described by Laver.[16] The compound 2'-(4-methylumbelliferyl)-α-D-acetylneuraminic acid (MUNANA) is the substrate of NA. And cleavage of this substrate by NA produces a fluorescent product, which can emit an emission wavelength of 460 nm with an excitation wavelength of 355 nm. The intensity of fluorescence can reflect the activity of NA sensitively.

In the enzyme reaction system, there were 30 μL of the enzyme in 33 mmol/L MES buffer (pH 3.5), 10 μL of 4 mmol/L $CaCl_2$, 20 μL of 20 μmol/L MUNANA, and 30 μL water in a 96-well microplate. The terminal volume was 100 μL. After 10 min at 37 °C, 150 μL of 14 mmol/L NaOH in 83% ethanol was added to 0.1 mL of the reaction mixture to terminate the reaction. The intensity of fluorescence was quantitated in Fluostar Galaxy (excitation, 360 nm; emission, 450 nm), and substrate blanks were subtracted from the sample readings. The IC_{50} was calculated by plotting percent inhibition versus the inhibitor concentration and determination of each point was performed in duplicate.

6.2. Chemistry: General procedures

All reactions except those in aqueous media were carried out by standard techniques for the exclusion of moisture. 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. ^{1}H NMR spectra were determined on a Brucker Avance 600 spectrometer using TMS as an internal standard. ESI-MS were determined on an API 4000 spectrometer. Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Anhydrous reactions were carried out in oven-dried glassware under a nitrogen atmosphere.

6.2.1. (2*S*,4*R*)-Methyl-4-hydroxypyrrolidine-2-carboxylate hydrochloride (3). The title compound was prepared as described by Jordis in (1*S*,4*S*)-2-thia-5-azabicyclo-[2.2.1]heptane.[17]

6.2.2. (2*S*,3*S*)-2-(*tert*-Butoxycarbonyl)-3-methylpentanoic acid (*N*-Boc-L-isoleucine) (4). The title compound was prepared as described by Haaina.[18]

6.2.3. (2*S*,4*R*)-Methyl-1-((3*S*)-2-(*tert*-butoxycarbonyl)-3-methylpentanoyl)-4-hydroxypyrrolidine-2-carboxylate(5a).[19] A solution of DCC (4.48 g, 21.6 mmol) in 30 mL anhyd THF was added dropwise to a solution of 4.62 g *N*-Boc-L-isoleucine (20 mmol) and 2.92 g HOBt (21.6 mmol) in 60 mL of anhyd THF at 0 °C for a period of about 20 h to get the HOBt active ester. Then the urea which was separated by filtration and the filtrate would be used in the next step.

Compound (**3**) was suspended in anhydrous THF, and 2.02 g *N*-methylmorpholine (NMM) (20 mmol) was added. After half-an-hour, the filtrate was added. The reaction mixture was stirred at room temperature overnight. The dicyclohexylurea (DCU) was filtered off and THF was evaporated in vacuo. The residues obtained after evaporation were dissolved in 150 mL EtOAc and the DCU was filtered off. The filtrate was washed with 10% citric acid, brine, and saturated $NaHCO_3$ solution, and then was dried with $NaSO_4$. The solvent was evaporated to give a residue that was chromatographed on a silica column to give the title dipeptide **5a** (yield: 83.2%) as an oil. ESI-MS *m*/*z* 359.6 (M+H); ^{1}H NMR (DMSO-d_6, ppm): δ 1.40 (s, 9H); 0.94 (d, J = 6.6 Hz, 3H); 0.97 (d, J = 6.5 Hz, 3H); 3.71 (s, 3H); 4.64 (t,J = 8.3 Hz, 1H); 1.50 (m, 2H); 1.74 (m, 1H); 2.33 (m, 1H); 1.99 (m, 1H); 3.06 (br, 1H); 3.69 dd, J = 3.7, 10.9 Hz, 1H); 3.97 (d, J = 10.9 Hz, 1H); 5.22 (d, J = 8.5 Hz, 1H); 4.40 (m, 1H); 4.55 (s, 1H); 6.80 (d, J = 8.3 Hz, 1H).

6.2.3.1. Methyl-1-(2-(*tert*-butoxycarbonyl)-3-phenylpropanoyl)-4-hydroxypyrrolidine-2-carboxylate (6a). Yield: 81.7%, ESI-MS 393.3 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.30 (s, 9H); 3.62 (s, 3H); 1.89 (m, 1H); 2.06 (m, 1H); 2.69 (m, 1H); 2.78 (m, 1H); 4.03 (d, J = 7.1 Hz, 1H); 4.06 (d, J = 5.7 Hz, 1H); 4.35(m, 1H); 4.36 (m, 1H); 5.21 (t, J = 3.7 Hz, 1H); 6.92 (m, 1H); 7.18 (m, 2H); 7.28 (m, 2H); 6.92 (d, J = 7.6 Hz, 1H).

6.2.3.2. Methyl-1-(2-(*tert*-butoxycarbonyl)-4-methylpentanoyl)-4-hydroxypyrrolidine-2-carboxylate (7a). Yield: 80.5%, ESI-MS: 359.6 (M+H). ^{1}H NMR (DMSO-d_6): δ 1.40 (s, 9H); 0.95 (d, J = 6.7 Hz, 3H); 0.97 (d, J = 6.5 Hz, 3H); 3.72 (s, 3H); 4.65 (t, J = 8.3 Hz, 1H); 1.50 (m, 2H); 1.74 (m, 1H); 2.34 (m, 1H); 1.99 (m, 1H); 3.06 (br, 1H); 3.69 (dd, J = 3.7 Hz, 10.9 Hz, 1H); 3.97 (d, J = 10.9 Hz, 1H); 5.23 (d, J = 8.5 Hz, 1H); 4.40 (m, 1H); 4.55 (s, 1H); 6.81 (d, J = 8.3 Hz, 1H).

6.2.3.3. Methyl-1-(2-(*tert*-butoxycarbonyl)-3-methylbutanoyl)-4-hydroxypyrrolidine-2-carboxylate (8a). Yield: 79.9%, ESI-MS 345.5 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.38 (s, 9H); 0.84 (d, J = 6.5 Hz, 3H); 0.89 (d, J = 6.6 Hz, 3H); 1.91 (m, 2H); 2.09 (m, 1H); 3.60 (s, 3H); 3.65 (m, 2H); 4.34 (t, J = 7.8 Hz, 1H); 4.03 (m, 1H); 6.68 (d, J = 8.7 Hz, 1H).

6.2.3.4. Methyl-1-(2-(*tert*-butoxycarbonyl)propanoyl)-4-hydroxypyrrolidine-2-carboxylate (9a). Yield: 75.6%, ESI-MS 317.5 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.37 (s, 9H); 1.14 (d,J = 7.0 Hz, 3H); 1.91 (m, 1H); 2.10 (m, 1H); 3.59 (s, 3H); 4.03 (q,J = 7.1 Hz, 1H); 4.23 (t,J = 7.2 Hz, 1H); 4.34 (m, 2H); 5.19 (m, 1H); 6.89 (m, J = 7.7 Hz, 1H).

6.2.3.5. Methyl-1-(2-(*tert*-butoxycarbonyl)-4-(methylthio)butanoyl)-4-hydroxypyrrolidine-2-carboxylate (10a). Yield: 82.5%, ESI-MS 377.5 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.38 (s, 9H); 1.91 (s, 3H); 3.58 (s, 3H); 1.78 (m,

1H); 2.11 (m, 1H); 2.46 (m, 1H); 2.51 (m, 1H); 2.04 (m, 2H); 3.71 (m, 1H); 4.35 (m, 1H); 5.19 (m, 1H); 4.03 (q, J = 7.1 Hz, 1H); 4.27 (t, J = 8.0 Hz, 1H); 6.96 (d, J = 8.6 Hz, 1H).

6.2.3.6. Methyl-1-(2-(2-(2,6-dichlorophenylamino)-phenyl)acetyl)-4-hydroxypyrrolidine-2-carboxylate (11a). Yield: 83.7%, mp = 99.8–101.2 °C, ESI-MS 423.5 (M+H), ^{1}H NMR (DMSO-d_6): δ 3.609 (s, 3H); 1.99 (m, 1H); 2.09 (m, 1H); 3.34 (s, 2H); 3.78 (m, 2H); 4.38 (t, J = 7.8 Hz, 1H); 5.28 (m,1H); 6.29 (m,1H); 6.87 (t,J = 7.5, 7.3 Hz, H); 7.06 (dt, J = 6.9, 7.2, 2.3 Hz, 1H); 7.16 (q, J = 8.1 Hz, 1H); 7.25 (dd,J = 7.2, 0.69 Hz, 1H); 7.51 (d, J = 8.1 Hz, 1H); 7.54 (s, 1H).

6.2.4. (2*S*,4*R*)-Methyl-1-((3*S*)-2-(*tert*-butoxycarbonyl)-3-methylpentanoyl)-4-hydroxypyrrolidine-2-carboxylate (5a1). To a solution of **5a** (7.16 g, 20 mmol) in 100 mL CH_2Cl_2 at 0 °C was added Et_3N (3.5 mL, 25 mmol) followed by dropwise addition of methanesulfonyl chloride (1.7 mL, 22 mmol). The reaction mixture was stirred at 0 °C for 45 min and warmed to room temperature with stirring for 4 h. The following mixture was diluted with 50 mL CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$ solution, H_2O, and brine in turn, dried with Na_2SO_4, filtered, and evaporated to afford crude mesylate **5a1**, which was of suitable purity to use directly in the next step (yield: 82.3%).

6.2.5. (2*S*,4*S*)-Methyl-4-azido-1-((3*S*)-2-(*tert*-butoxycarbonyl)-3-methylpentanoyl)pyrrolidine-2-carboxylate (5a2).[20] To a solution of **5a1** (8.72 g, 20 mmol) in 30 mL anhyd DMF was added ground sodium azide (1.39 g, 21.4 mmol), and the reaction mixture was heated at 55 °C for 10 h. The reaction mixture was cooled to room temperature and then partitioned between water and EtOAc. The organic layer was washed with brine, dried with Na_2SO_4, filtered, and evaporated to get pale yellow oil (yield: 63.5%).

6.2.6. (2*S*,4*S*)-Methyl-4-amino-1-((3S)-2-(*tert*-butoxycarbonyl)-3-methylpentanoyl)pyrrolidine-2-carboxylate (5d).[21] In hydrogen atmosphere, 7.66 g compound **5a2** (20 mmol) was dissolved in anhyd methanol, and 5% Pd/$CaCO_3$ (0.77 g) was added. The resulting solution was stirred for 10 h under 760 mm Hg pressure, during which the hydrogen was bubbled intermittently. The resulting mixture was filtered and the filtrate was evaporated to give pale yellow oil, which was purified by flash chromatography on silica gel to give **5d** as pale yellow oil (yield: 60.8%). ESI-MS m/z 358.5 (M+H); ^{1}H NMR (DMSO-d_6, ppm): δ 1.36 (s, 9H); 0.82 (t, J = 7.5 Hz, 3H); 0.88 (d,J = 6.6 Hz, 3H); 1.09 (m, 2H); 1.56 (m, 1H); 1.71 (m, 1H); 2.33 (m, 1H); 3.17 (m, 2H); 3.42 (m, 1H); 4.01 (m, 1H); 4.25 (t, J = 8.1 Hz, 1H); 6.87 (d,J = 8.7 Hz, 1H).

6.2.6.1. Methyl-4-amino-1-(2-(*tert*-butoxycarbonyl)-3-phenylpropanoyl)pyrrolidine-2-carboxylate (6d). Yield: 60.5%, ESI-MS 392.5 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.28 (s, 9H); 3.61 (s, 3H); 1.59 (m, 1H); 2.40 (m, 1H); 2.75 (m, 1H); 2.91 (m, 1H); 3.76 (d, J = 5.8 Hz, 1H); 3.84 (d, J = 4.4 Hz, 1H); 4.30 (t, J = 7.8 Hz, 1H); 4.10 (m, 1H); 4.37 (m, 1H); 7.08 (m, 1H); 7.19 (m, 2H); 7.29 (m, 2H); 6.99 (d, J = 8.3 Hz, 1H).

6.2.6.2. Methyl-4-amino-1-(2-(*tert*-butoxycarbonyl)-4-methylpentanoyl)pyrrolidine-2-carboxylate (7d). Yield: 61.2%, ESI-MS 358.5 (M+H), ^{1}H NMR (DMSO-d_6): δ1.36 (s, 9H); 0.88 (d, J = 7.9 Hz, 3H); 0.90 (d, J = 4.2 Hz, 3H); 3.59 (s, 3H); 1.41 (m, 2H); 1.52 (m, 1H); 1.55 (m, 1H); 2.34 (m, CH_2, 1H); 4.26 (t, CH, J = 8.1 Hz, 1H); 3.88 (dd, CH, J = 3.7 Hz, 10.6 Hz, 1H); 4.20 (m, CH, 1H); 3.43 (d, CH_2, J = 7.0 Hz, 1H); 6.90 (d, CH_2, J = 8.1 Hz, 1H); 6.90 (d, NH, J = 8.1 Hz, 1H).

6.2.6.3. Methyl-4-amino-1-(2-(*tert*-butoxycarbonyl)-3-methylbutanoyl)pyrrolidine-2-carboxylate (8d). Yield: 59.8%, ESI-MS 344.6 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.36 (s, 9H); 0.86 (d, J = 6.5 Hz, 3H); 0.92 (d, J = 6.6 Hz, 3H); 3.60 (s, 3H); 1.54 (m, 1H); 1.94 (m, 1H); 2.35 (m, 1H); 3.16 (m, 1H); 3.43 (m, H); 3.99 (m, 2H); 4.26 (t, J = 8.2 Hz, 1H); 6.75 (d, J = 8.4 Hz, 1H).

6.2.6.4. Methyl-4-amino-1-(2-(*tert*-butoxycarbonyl)-propanoyl)pyrrolidine-2-carboxylate (9d). Yield: 61.3%, ESI-MS 316.5 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.36 (s, 9H); 1.16 (d, J = 6.8 Hz, 3H); 3.60 (s, 3H); 3.80 (q, J = 4.3 Hz, 1H); 4.21 (t, J = 7.5 Hz, 1H); 2.29 (m, 2H); 3.60 (m, 2H); 4.05 (m, 1H); 6.90 (d, J = 7.4 Hz, 1H).

6.2.6.5. Methyl-4-amino-1-(2-(*tert*-butoxycarbonyl)-4-(methylthio)butanoyl)pyrrolidine-2-carboxylate (10d). Yield: 60.5%, ESI-MS 376.5 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.36 (s, 3H); 2.06 (s, 3H); 3.17 (s, 3H); 1.55 (m, 2H); 1.80 (m, 2H); 2.32 (m, 2H); 3.30 (m, 1H); 3.62 (m, 2H); 3.92 (m, 1H); 4.28 (t, J = 8.1 Hz, 1H); 7.09 (d, J = 7.5 Hz, 1H).

6.2.6.6. Methyl-4-amino-1-(2-(2-(2,6-dichlorophenylamino)phenyl)acetyl)pyrrolidine-2-carboxylate (11d). Yield: 63.7%, mp = 59.8–61.4 °C, ESI-MS: 422.5 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.60 (m, 1H); 2.35 (m, 1H); 3.59 (s, 3H); 3.76 (s, 2H); 3.25 (m, 1H); 3.49 (m, 1H); 3.92 (m, 1H); 4.31 (t, J = 7.8 Hz, 1H); 6.29 (m, 1H); 6.86 (t, J = 7.0 Hz, 1H); 7.05 (t, J = 7.3 Hz, 1H); 7.15 (q, J = 8.1 Hz, 1H); 7.23 (d, J = 7.2 Hz, 1H); 7.50 (d, J = 8.2 Hz, 2H); 7.73 (s, 1H).

6.2.7. 1-(2-(*tert*-Butoxycarbonyl)-4-methylpentanoyl)-4-hydroxypyrrolidine-2-carboxylic acid (5b).[22] Compound **5a** (7.66 g, 20 mmol) was dissolved in 100 mL MeOH, and 20 mL of 2 mol/L NaOH was added over 5min with good stirring. This was allowed to stir for 24h at room temperature. The reaction mixture was filtered and adjusted to pH 5–6 with 80% acetic acid/water. The residual solution was evaporated to remove MeOH and then was acidified to pH 2–3 with 40% citric acid solution and extracted with EtOAc. The extract was washed with saturated NaCl, dried over Na_2SO_4. The solvent was removed in vacuo to obtain **5b** (yield 79.3%, mp = 79–80 °C). ESI-MS m/z 345.4 (M+H); ^{1}H NMR (DMSO-d_6, ppm): δ 1.37 (s, 9H); 0.80 (t, 3H); 0.87 (d, J = 6.6 Hz, 3H); 1.04 (m, 2H);1.68 (m, 1H); 1.88 (m, 1H); 2.08 (m, 1H); 3.64 (m, 1H); 4.05 (m,

2H); 5.17 (m, 1H); 4.23 (t, J = 8.1 Hz, 1H); 6.78 (d, J = 9.0 Hz, 1H).

6.2.7.1. 1-(2-(*tert*-Butoxycarbonyl)-3-phenylpropanoyl)-4-hydroxypyrrolidine-2-carboxylic acid (6b). Yield: 79.5%, mp = 96–97 °C, ESI-MS 379.6 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.29 (s, 9H); 1.91 (m, 1H); 2.10 (m, 1H); 2.75 (m, 1H); 2.87 (m, 1H); 3.56 (d, J = 3.6 Hz, 1H); 3.62 (d, J = 4.5 Hz, 1H); 4.29 (m, 1H); 4.34 (m, 1H); 5.16 (t, J = 3.7 Hz, 1H); 6.94 (m, 1H); 7.19 (m, 2H); 7.27 (m, 2H); 12.42 (s, 1H); 6.94 (d, J = 8.5 Hz, 1H).

6.2.7.2. 1-(2-(*tert*-Butoxycarbonyl)-4-methylpentanoyl)-4-hydroxypyrrolidine-2-carboxylic acid (7b). Yield: 78.7%, mp = 167–168 °C, ESI-MS: 345.5 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.42 (s, 9H); 0.95 (d, J = 6.6 Hz, 3H); 0.97 (d, J = 6.5 Hz, 3H); 4.77 (t, J = 8.3 Hz, 1H); 1.53 (m, 2H); 1.57 (m, 1H); 2.35 (m, 1H); 1.68 (m, 1H); 3.60 (dd, J = 3.5, 11.8 Hz, 1H); 4.21 (d, J = 11.2 Hz, 1H); 5.21 (d, J = 8.1 Hz, 1H); 4.46 (m, 1H); 4.55 (s, 1H); 6.85 (d, J = 8.9 Hz, 1H).

6.2.7.3. 1-(2-(*tert*-Butoxycarbonyl)-3-methylbutanoyl)-4-hydroxypyrrolidine-2-carboxylic acid (8b). Yield: 77.9%, mp = 171.9–176.3 °C, ESI-MS: 331.5 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.37 (s, 9H); 0.83 (d, J = 6.6 Hz, 3H); 0.89 (d, J = 6.7 Hz, 3H); 1.90 (m, 2H); 2.08 (m, 1H); 3.61 (m, 2H); 4.03 (m, 1H); 4.26 (t, J = 8.1 Hz, 1H); 5.13 (m, 1H); 6.64 (d, J = 8.7 Hz, 1H); 12.35 (s, 1H).

6.2.7.4. 1-(2-(*tert*-Butoxycarbonyl)propanoyl)-4-hydroxypyrrolidine-2-carboxylic acid (9b). Yield: 76.5%, mp = 169.7–170.4 °C, ESI-MS: 303.6 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.36 (s, 9H); 1.13 (d, 3H); 1.90 (m, 1H); 2.06 (m, 1H); 3.59 (q, J = 7.4 Hz, 1H); 4.26 (m, 2H); 4.34 (t, J = 2.2 Hz, 1H); 5.15 (m, 1H); 6.87 (d,J = 7.6 Hz, 1H).

6.2.7.5. 1-(2-(*tert*-Butoxycarbonyl)-4-(methylthio)butanoyl)-4-hydroxypyrrolidine-2-carboxylic acid (10b). Yield: 79.1%, mp = 132.6–133.9 °C, ESI-MS: 363.6 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.37 (s, 9H); 2.04 (s, 3H); 1.58 (m, 2H); 1.77 (m, 2H); 2.04 (m, 2H); 3.24 (m, 1H); 3.63 (m, 2H); 4.29 (t, J = 7.5 Hz, 1H); 5.20 (m, 1H); 7.03 (d, J = 6.6 Hz, 1H).

6.2.7.6. 1-(2-(2-(2,6-Dichlorophenylamino)phenyl)acetyl)-4-hydroxypyrrolidine-2-carboxylic acid (11b). Yield: 82.3%, mp = 116–117 °C, ESI-MS: 409.6 (M+H), ^{1}H NMR(DMSO-d_6): δ 1.94 (m, 1H); 2.12 (m, 1H); 3.30 (s, 2H); 3.72 (m, 2H); 4.30 (t, J = 7.8 Hz, 1H); 5.20 (m, 1H); 6.29 (m, 1H); 6.85 (t, J = 7.4 Hz, 1H); 7.05 (dt,J = 8.2, 7.6, 1.3 Hz, 1H); 7.15 (q, J = 8.1 Hz, 1H); 7.24 (dd, J = 6.9, 0.9;Hz, 1H); 7.50 (d, J = 7.9 Hz, 1H); 7.64 (s, 1H).

6.2.8. *tert*-Butyl-1-(4-hydroxy-2-(hydroxycarbamoyl)-pyrrolidin-1-yl)-3-methyl-1-oxopentan-2-ylcarbamate (5c).[23] Compound **5a** (7.66 g, 20 mmol) was dissolved in 100 mL anhyd MeOH and 15 mL MeOH with NH_2OK (prepared by Fieser and Fieser. Vol. 1, p 478). The resulting solution was stirred at 65 °C for 10 h, then 7.5 g silica gel was added and evaporated to give pale yellow powder, which was purified by column chromatography to provide 4.67 g of compound **5c** as a white solid, which appeared red in $FeCl_3$. Yield: 62.5%, mp = 94 –95 °C, ESI-MS m/z 360.6: (M+H); ^{1}H NMR (DMSO-d_6, ppm): δ 1.36 (s, 9H); 0.80 (t, J = 7.2 Hz, 3H); 0.83 (d, J = 6.4 Hz, 3H); 1.05 (m, 2H); 1.68 (m, 1H); 1.91 (m, 1H); 2.07 (m, 1H); 3.61 (m, 1H); 3.99 (m, 1H); 4.19 (m, 1H); 5.07 (m, 1H); 4.33 (t, J = 7.3 Hz, 1H); 6.68 (d, J = 8.7 Hz, 1H).

6.2.8.1. *tert*-Butyl-1-(4-hydroxy-2-(hydroxycarbamoyl)-pyrrolidin-1-yl)-1-oxo-3-phenylpropan-2-ylcarbamate (6c). Yield: 62.3%, mp = 104–105 °C, ESI-MS: 394.5 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.28 (s, 9H); 1.90 (m, 1H); 1.96 (m, 1H); 2.70 (m, 1H); 2.87 (m, 1H); 3.52 (d, J = 4.6 Hz, 1H); 3.63 (d, J = 4.5 Hz, 1H); 4.24 (t, J = 7.5 Hz, 1H); 4.36 (m, 1H); 5.11 (m, 1H); 6.93 (m, 1H); 7.21 (m, 2H); 7.28 (m, 2H); 8.80 (s, 1H); 10.53 (s, 1H); 6.93 (d, J = 8.4 Hz, 1H).

6.2.8.2. *tert*-Butyl-1-(4-hydroxy-2-(hydroxycarbamoyl)-pyrrolidin-1-yl)-4-methyl-1-oxopentan-2-ylcarbamate (7c). Yield: 62.1%, mp = 100–101 °C, ESI-MS: 360.6 (M+H), ^{1}H NMR (DMSO-d_6): δ1.42 (s, 9H); 0.93 (d, J = 6.6 Hz, 3H); 0.96 (d, J = 6.4 Hz, 3H); 1.52 (m, 2H); 4.57 (t, J = 8.3 Hz, 1H); 1.72 (m, 1H); 2.78 (m, 1H); 2.27 (m, 1H); 3.87 (dd, J = 3.6, 9.6 Hz, 1H); 4.41 (s, 1H); 3.74 (m, 1H); 3.98 (d, J = 11.0 Hz, 1H); 5.77 (d, J = 8.6 Hz, 1H); 6.79 (d, J = 8.2 Hz, 1H).

6.2.8.3. *tert*-Butyl-1-(4-hydroxy-2-(hydroxycarbamoyl)-pyrrolidin-1-yl)-3-methyl-1-oxobutan-2-ylcarbamate (8c). Yield: 60.7%, mp = 90–91 °C, ESI-MS 346.4: (M+H), ^{1}H NMR (DMSO-d_6): δ 1.37 (s, 9H); 0.82 (d, J = 6.5 Hz, 3H); 0.89 (d, J = 6.5 Hz, 3H); 1.86 (m, 2H); 1.91 (m, 1H); 3.60 (m, 2H); 3.99 (m, 1H); 4.20 (t, J = 7.8 Hz, 1H); 5.07 (m, 1H); 6.60 (d, J = 8.6 Hz, 1H); 8.76 (s, 1H); 10.52 (s, 1H).

6.2.8.4. *tert*-Butyl-1-(4-hydroxy-2-(hydroxycarbamoyl)-pyrrolidin-1-yl)-1-oxopropan-2-ylcarbamate (9c). Yield: 59.8%, mp = 98–99 °C, ESI-MS 318.5: (M+H), ^{1}H NMR (DMSO-d_6): δ 1.36 (s, 9H); 1.14 (d, J = 7.0 Hz, 3H); 1.87 (m, 1H); 1.98 (m, 1H); 4.02 (q, J = 7.2 Hz, 1H); 4.20 (t, J = 7.5 Hz, 1H); 3.48 (m, 1H); 3.60 (m, 1H); 4.36 (m, 1H); 6.87 (d, J = 7.8 Hz, 1H); 10.35 (s, 1H); 10.51 (s, 1H).

6.2.8.5. *tert*-Butyl-1-(4-hydroxy-2-(hydroxycarbamoyl)-pyrrolidin-1-yl)-4-(methylthio)-1-oxobutan-2-ylcarbamate (10c). Yield: 60.7%, mp = 66 –67 °C, ESI-MS 378.6: (M+H), ^{1}H NMR (DMSO-d_6): δ 1.38 (s, 9H); 2.09 (s, 3H); 1.86 (m, 2H); 1.98 (m, 3H); 2.50 (m, 3H); 3.63 (m, 1H); 4.31 (m, 2H); 4.20 (t, J = 7.8 Hz, 1H); 5.14 (m, 1H); 6.98 (d, J = 8.1 Hz, 1H); 8.83 (s, 1H); 10.58 (s, 1H).

6.2.8.6. 1-(2-(2-(2,6-Dichlorophenylamino)phenyl)acetyl)-*N*,4-dihydroxypyrrolidine-2-carboxamide (11c). Yield: 63.7%, mp = 102.6–104.2 °C, ESI-MS: 424.5 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.75 (m, 1H); 1.93 (m, 1H); 3.31 (s, 2H); 3.74 (m, 2H); 4.26 (t, J = 7.5 Hz, 1H); 5.15 (d,

J = 3.9 Hz, 1H); 6.28 (m, 1H); 6.84 (t, J = 7.3 Hz, 1H); 7.04 (dt, J = 7.8, 8.1, 1.3 Hz, 1H); 7.23 (dd, J = 7.3, 0.9 Hz, 1H); 7.50 (d, J = 8.0 Hz, 2H); 7.72 (s, 1H).

6.2.9. (2*S*,4*S*)-4-Azido-1-((3*S*)-2-(*tert*-butoxycarbonyl)-3-methylpentanoyl)pyrrolidine-2-carboxylic acid (5d1). Compound **5d** was converted to compound **5d1** as described for compound **5b** (yield 77.8%).

6.2.10. (2*S*,4*S*)-4-Amino-1-((3*S*)-2-(*tert*-butoxycarbonyl)-3-methylpentanoyl)pyrrolidine-2-carboxylic acid (5e). Compound **5d1** was converted to compound **5e** as described for compound **5d**. Yield: 59.7%, mp = 160.7–161.8 °C, ESI-MS m/z 344.6: (M+H); ^{1}H NMR (DMSO-d_6, ppm): δ 1.36 (s, 9H); 0.81 (t,J = 7.2 Hz, 3H); 0.86 (d, J = 6.5 Hz, 3H); 1.11 (m, 2H); 1.69 (m, 1H); 1.88 (m, 1H); 2.27 (m, 1H); 3.85 (m, 2H); 4.05 (m, 1H); 3.95 (t, J = 8.7 Hz, 1H); 5.76 (m, 1H); 6.93 (d, J = 8.7 Hz, 1H).

6.2.10.1. 4-Amino-1-(2-(*tert*-butoxycarbonyl)-3-phenylpropanoyl)pyrrolidine-2-carboxylic acid (6e). Yield: 61.2%, mp = 175–176 °C, ESI-MS: 376.4 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.29 (s, 9H); 1.97 (m, 1H); 2.23 (m, 1H); 2.74 (m, 1H); 2.91 (m, 1H); 3.51 (d, J = 5.5 Hz, 1H); 3.85 (d, J = 5.1 Hz, 1H); 3.79 (m, 1H); 4.09 (m, 1H); 4.54 (t, J = 7.7 Hz, 1H); 7.04 (m, 1H); 7.19 (m, 2H); 7.26 (m, 2H); 8.90 (br, 2H); 7.04 (d, J = 8.5 Hz, 1H).

6.2.10.2. 4-Amino-1-(2-(*tert*-butoxycarbonyl)-4-methylpentanoyl)pyrrolidine-2-carboxylic acid (7e). Yield: 60.7%, mp = 181–182 °C, ESI-MS: 342.5 (M−H), ^{1}H NMR (DMSO-d_6): δ 1.36 (s, 9H); 0.85 (d, J = 3.4 Hz, 3H); 0.89 (d, J = 6.3 Hz, 3H); 1.62 (m, 2H); 2.24 (m, 1H); 4.50 (t, J = 5.1 Hz, 1H); 1.89 (m, 2H); 3.66 (m, 1H); 4.07 (dd, J = 8.9, 18.3 Hz, 1H); 3.82 (m, 1H); 3.76 (d, J = 5.4 Hz, 1H); 6.95 (d, J = 12.3 Hz, 1H); 8.81 (br, 2H); 6.91 (d, J = 8.2 Hz, 1H).

6.2.10.3. 4-Amino-1-(2-(*tert*-butoxycarbonyl)-3-methylbutanoyl)pyrrolidine-2-carboxylic acid (8e). Yield: 59.9%, mp = 235–236 °C, ESI-MS: 330.5 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.36 (s, 9H); 0.87 (d, J = 4.7 Hz, 3H); 0.91 (d, J = 6.6 Hz, 3H); 1.91 (m, 2H); 2.21 (m, 1H); 3.61 (m, 1H); 3.77 (m, 2H); 4.05 (m, 1H); 3.90 (t, J = 8.3 Hz, 1H); 6.80 (d, J = 8.4 Hz, 1H); 8.74 (br, 2H).

6.2.10.4. 4-Amino-1-(2-(*tert*-butoxycarbonyl)propanoyl)pyrrolidine-2-carboxylic acid (9e). Yield: 59.6%, mp = 248–249 °C, ESI-MS: 302.8 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.36 (s, 9H); 1.15 (d, J = 6.7 Hz, 3H); 3.79 (q, J = 9.3 Hz, 1H); 4.17 (t, J = 7.8 Hz, 1H); 2.31 (m, 2H); 3.39 (m, 2H); 3.84 (m, 1H); 7.02 (d, J = 7.0 Hz, 1H).

6.2.10.5. 4-Amino-1-(2-(*tert*-butoxycarbonyl)-4-(methylthio)butanoyl)pyrrolidine-2-carboxylic acid (10e). Yield: 61.7%, mp = 152.4–153.7 °C, ESI-MS: 362.6 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.36 (s, 9H); 2.05 (s, 3H); 1.84 (m, 2H); 1.94 (m, 1H); 2.29 (m, 1H); 2.40 (m, 2H); 3.55 (m, 1H); 3.75 (m, 2H); 4.16 (m, 1H); 4.26 (t, J = 6.1 Hz, 1H); 7.11 (d, J = 8.1 Hz, 1H).

6.2.10.6. 4-Amino-1-(2-(2-(2,6-dichlorophenylamino)-phenyl)acetyl)pyrrolidine-2-carboxylic acid (11e). Yield: 62.5%, mp = 196–197 °C, ESI-MS: 408.5 (M+H), ^{1}H NMR (DMSO-d_6): δ 2.11 (d, J = 14.1 Hz, 1H); 4.41 (d, J = 9.2 Hz, 1H); 3.53 (dd, J = 5.2, 13.4 Hz, 2H); 3.76 (s, 2H); 3.70 (m, 1H); 3.75 (m, 1H); 6.29 (d, J = 7.9 Hz, 1H); 6.82 (t, J = 6.8 Hz, 1H); 7.03 (t, J = 7.4 Hz, 1H); 7.15 (q, J = 8.1 Hz, 1H); 7.23 (d, J = 6.4 Hz, 1H); 7.50 (d, J = 4.4 Hz, 2H); 8.18 (s, 1H).

6.2.11. *tert*-Butyl-1-(4-amino-2-(hydroxycarbamoyl)pyrrolidin-1-yl)-3-methyl-1-oxopentan-2-ylcarbamate (5f). Compound **5d** was converted to compound **5f** as described for compound **5c**. Yield 63.4%, mp = 115.8–116.9 °C, ESI-MS: m/z 359.6 (M+H); ^{1}H NMR (DMSO-d_6,ppm): δ 1.36 (s, 9H); 0.80 (t, J = 7.2 Hz, 3H); 0.86 (d, J = 6.6 Hz, 3H); 1.09 (m, 2H); 1.48 (m, 1H); 1.66 (m, 1H); 2.30 (m, 1H); 3.39 (m, 1H); 3.56 (m, 1H); 3.96 (m, 2H); 4.13 (t, J = 6.2 Hz, 1H); 6.87 (d, J = 8.2 Hz, 1H).

6.2.11.1. *tert*-Butyl-1-(4-amino-2-(hydroxycarbamoyl)-pyrrolidin-1-yl)-1-oxo-3-phenylpropan-2-ylcarbamate (6f). Yield: 61.7%, mp = 119–120 °C, ESI-MS: 391.0 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.28 (s, 9H); 1.72 (m, 1H); 2.37 (m, 1H); 2.75 (m, 1H); 2.85 (m, 1H); 3.52 (d, J = 3.5 Hz, 1H); 3.85 (d, J = 5.6 Hz, 1H); 3.65 (m, 1H); 4.21 (m, 1H); 4.29 (t, J = 7.6 Hz, 1H); 7.09 (m, 1H); 7.19 (m, 2H); 7.25 (m, 2H); 7.09 (d, J = 8.2 Hz, 1H).

6.2.11.2. *tert*-Butyl-1-(4-amino-2-(hydroxycarbamoyl)-pyrrolidin-1-yl)-4-methyl-1-oxopentan-2-ylcarbamate (7f). Yield: 62.3%, mp = 126–127 °C, ESI-MS: 359.6 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.36 (s, 9H); 0.84 (d, J = 7.9 Hz, 3H); 0.88 (d, J = 4.21 Hz, 3H); 1.57 (m, 2H); 1.65 (m, 1H); 1.68 (m, 1H); 2.31 (m, 1H); 4.15 (t, J = 5.2 Hz, 1H); 3.85 (dd, J = 9.8, 9.9 Hz, 1H); 3.58 (m, 1H); 3.34 (d, J = 4.4 Hz, 1H); 3.58 (d, J = 5.3 Hz, 1H); 6.92 (d, J = 7.9 Hz, 1H).

6.2.11.3. *tert*-Butyl-1-(4-amino-2-(hydroxycarbamoyl)-pyrrolidin-1-yl)-3-methyl-1-oxobutan-2-ylcarbamate (8f). Yield: 61.5%, mp = 108–109 °C, ESI-MS: 345.6 (M+H), ^{1}H NMR (DMSO-d_6): δ1.36 (s, 9H); 0.85 (d, J = 6.5 Hz, 3H); 0.91 (d, J = 6.6 Hz, 3H); 1.89 (m, 2H); 2.23 (m, 1H); 3.23 (m, 1H); 3.37 (m, 1H); 3.92 (m, 2H); 4.07 (t, J = 7.4 Hz, 1H); 6.73 (d, J = 8.2 Hz, 1H).

6.2.11.4. *tert*-Butyl-1-(4-amino-2-(hydroxycarbamoyl)-pyrrolidin-1-yl)-1-oxopropan-2-ylcarbamate (9f). Yield: 61.2%, mp = 144–146 °C, ESI-MS: 317.8 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.36 (s, 9H); 1.15 (d, J = 6.7 Hz, 3H); 3.80 (q, J = 9.2 Hz, 1H); 4.27 (t, J = 8.0 Hz, 1H); 2.35 (m, 2H); 3.44 (m, 2H); 3.85 (m, 1H); 7.03 (d, J = 7.0 Hz, 1H).

6.2.11.5. *tert*-Butyl-1-(4-amino-2-(hydroxycarbamoyl)-pyrrolidin-1-yl)-4-(methylthio)-1-oxobutan-2-ylcarbamate (10f). Yield: 60.7%, mp = 108–109 °C, ESI-MS: 377.7 (M+H), ^{1}H NMR (DMSO-d_6): δ 1.36 (s, 9H); 2.05 (s, 3H); 1.75 (m, 2H); 2.01 (m, 2H); 2.47 (m, 2H); 3.61 (m, 1H); 3.71 (m, 2H); 4.30 (m, 1H); 4.20 (t, J = 4.1 Hz, 1H); 7.04 (d, J = 7.3 Hz, 1H).

6.2.11.6. 4-Amino-1-(2-(2-(2,6-dichlorophenylamino)-phenyl)acetyl)-*N*-hydroxypyrrolidine-2-carboxamide (11f). Yield: 65.6%, mp = 159–160 °C, ESI-MS: 423.5 (M+H), ^{1}H NMR (DMSO-d_6): δ 2.03 (d, J = 14.0 Hz, 1H); 4.39 (d, J = 9.1 Hz, 1H); 3.50 (dd, J = 5.1, 13.2 Hz, 2H); 3.75 (s, 2H); 3.71 (m, 1H); 3.72 (m, 1H); 6.31 (d, J = 7.9 Hz, 1H); 6.83 (t, J = 6.8 Hz, 1H); 7.05 (t, J = 7.2 Hz, 1H); 7.17 (q, J = 8.0 Hz, 1H); 7.28 (d, J = 6.2 Hz, 1H); 7.50 (d, J = 4.2 Hz, 2H); 8.20 (s, 1H).

6.2.12. 1-(2-Amino-3-methylpentanoyl)-4-hydroxypyrrolidine-2-carboxylic acid (5g).[24] 6.88 g compound **5b** (20 mmol) was dissolved in 20 mL HCl–EtOAc (3 mol/L). After 30 min, the solution was removed in vacuo. The residue was washed with ether to get 3.08 g of compound **5g** as a white solid. Yield: 63.2%, mp = 189.7–191.9 °C, ESI-MS: *m*/*z* 245.4 (M+H); ^{1}H NMR (DMSO-d_6, ppm): δ 0.86 (t, J = 7.36 Hz, 3H); 0.99 (d, J = 6.8 Hz, 3H); 1.15 (m, 1H); 1.67 (m, 1H); 2.17 (m, 1H); 1.88 (m, 2H); 3.55 (m, 1H); 3.70 (m, 1H); 4.02 (m, 1H); 5.30 (m, 1H); 4.35 (t, J = 8.6 Hz, 1H); 8.21 (s, 1H); 12.59 (s, 1H).

6.2.12.1. Methyl-1-(2-amino-4-methylpentanoyl)-4-hydroxypyrrolidine-2-carboxylate (6g). Yield: 65.7%, mp = 52.0–52.8 °C, ESI-MS: 259.4 (M+H), ^{1}H NMR (DMSO-d_6): δ 0.90 (d, J = 6.7 Hz, 3H); 0.92 (d, J = 6.5 Hz, 3H); 1.50 (m, 1H); 1.63 (m, 1H); 1.87 (m, 2H); 2.19 (m, 1H); 3.62 (s, 3H); 3.55 (m, 1H); 3.74 (m, 2H); 4.11 (m, 1H); 4.40 (t, J = 7.7 Hz, 1H); 8.40 (s, 1H).

6.2.12.2. 1-(2-Amino-4-methylpentanoyl)-4-hydroxypyrrolidine-2-carboxylic acid (7g). Yield: 62.3%, mp = 212–213 °C, ESI-MS: 245.6 (M+H), ^{1}H NMR (DMSO-d_6): δ 0.91 (d, J = 6.5 Hz, 3H); 0.93 (d, J = 6.4 Hz, 3H); 1.51 (m, 1H); 1.59 (m, 1H); 1.76 (m, 1H); 1.91 (m, 1H); 2.18 (m, 1H); 3.54 (m, 1H); 3.59 (m, 2H); 3.67 (m, 1H); 4.35 (t, J = 8.3 Hz, 1H); 8.34 (s, 1H).

6.2.12.3. Methyl-4-amino-1-(2-amino-4-methylpentanoyl)pyrrolidine-2-carboxylate (9g). Yield: 61.3%, mp = 199.3–201.8 °C, ESI-MS: 258.5 (M+H), ^{1}H NMR (DMSO-d_6): δ 0.91 (d, J = 6.0 Hz, 3H); 0.94 (d, J = 6.1 Hz, 3H); 3.66 (s, 3H); 1.52 (m, 1H); 1.59 (m, 1H); 1.83 (m, 1H); 1.97 (m, 1H); 2.69 (m, 1H); 3.48 (t, J = 9.3 Hz, 1H); 3.77 (m, 1H); 4.12 (m, 1H); 4.25 (m, 1H); 4.41 (t, J = 8.9 Hz, 1H); 8.40 (s, 2H); 8.80 (s, 2H).

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References and notes

1. Pennisi, E. *Science* **1995**, *270*, 1916–1917.
2. Heins, J. R.; Plamp, J. *S. D. J. Med.* **2004**, *74*, 3–7.
3. Wiley, D. C.; Skehel, J. J. *Annu. Rev. Biochem,* **1987**, *56*, 365–394.
4. Liu, C. G.; Eichelberger, M. C.; Compans, R. W. *J. Virol.* **1995**, *69*, 1099–1106.
5. Ghate, A. A.; Air, G. M. *Eur. J. Biochem.* **1998**, *25*, 320–327.
6. Stoll, V.; Stewart, K. D.; Maring, C. J. *Biochemistry* **2003**, *42*, 718–727.
7. Young, D.; Fowler, C.; Bush, K. *Philos. Trans. R. Soc. London B* **2001**, *356*, 1905–1913.
8. Hanessian, S.; Bayrekdarian, M.; Luo, X. H. *J. Am. Chem. Soc.* **2002**, *124*, 4716–4721.
9. Wang, G. T.; Chen, Y.; Wang, S. *J. Med. Chem.* **2001**, *44*, 1192–1201.
10. Li, Y. L.; Xu, W. F. *Bioorg. Med. Chem.* **2004**, *12*, 5171–5180.
11. Zhang, L.; Zhang, J.; Fang, H.; Wang, Q.; Xu, W. F. *Bioorg. Med. Chem.* **2006**, *14*, 8286–8294.
12. Chan, M. C. W.; Cheung, C. Y.; Chui, W. H. *Respir. Res.* **2005**, *6*, 135–149.
13. Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Pietranico, S.; Leahy EM, Salvino J. *J. Am. Chem. Soc.* **1993**, 12550–12568.
14. Morphy, R.; Rankovic, Z. *J. Med. Chem,* **2005**, *48*, 6523–6543.
15. Liu, A. L.; Cao, H. P.; Du, G. H. *Sci. China Ser. C* **2005**, *48*, 1–5.
16. Laver, W. G.; Coleman, P. M.; Webster, R. G.; hinshaw, V. S.; Air, G. M. *Virology* **1984**, *137*, 314–323.
17. Jordis, U.; Sauter, F.; Siddiqi, S. M. *Indian J. Chem. Sec. B* **1989**, *28*, 294–296.
18. Haaina, G.; Lohse, A.; Buchardt, O. *Angew. Chem., Int. Ed. Engl. Comm.* **1995**, *35*, 1939–1942.
19. Ishida, H.; Qi, Z.; Sokabe, M. *J. Org. Chem.* **2001**, *66*, 2978–2982.
20. Abraham, D. J.; Molotoff, M.; Sheh, L. *J. Med. Chem.* **1983**, 549–554.
21. Brown, H. C.; Salunkhe, A. M. *Tetrahedron Lett* **1995**, *36*, 2567–2570.
22. Webb, T. R.; Eigenbrot, C. *J. Org. Chem.* **1991**, *56*, 3009–3016.
23. Niclsen, B. B.; Larsen, I. K. *Acta Crystallogr* **1993**, *C49*, 810.
24. Cavelier, F.; Enjalbal, C. *Tetrahedron Lett.* **1996**, *37*, 5131–5134.