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Original article

Synthesis of di- and tripeptide analogues containing α-ketoamide as a new core structure for inhibition of HIV-1 protease

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Abstract – Di- and tripeptide analogues containing α-ketoamide as a new core structure and incorporating allophenylnorstatine (Apns) as a transition state mimic, were designed and synthesized in the hope of obtaining a novel structural type of HIV-1 protease inhibitors. The immediate precursor, Apns-Thz-NHBu^t was prepared by coupling of Boc-Apns with *N-tert*·butyl Thz-4-carboxamide hydrochloride. Removal of Boc group followed by coupling with the respective α-ketoacid residue (P2) gave the desired dipeptides (**8**–**12**) in almost quantitative yields. The α-keto tripeptides (**18**–**21**) were obtained by oxidation of the hydroxyl group of Apns (PI) in the appropriate tripeptide, iQOA-Val-Apns-(un)substituted Thz(Oxa)-NHBu^t with DMSO/DCC. Preliminary evaluation of the activity of the synthesized derivatives was determined as percentage of enzyme inhibition at 5 μM and 50 nM levels of the di- and tripeptides respectively. The α-ketoamides displayed a significant enhanced potency relative to their parent isosteres as inhibitors of HIV-1 protease and are shown to be a promising new core structure for the development of enzyme inhibitors. A quantitative approach was attempted, using an LFE model, correlating the effect of structural modification and HIV-1 protease inhibition activity of the prepared dipeptides. The result indicates the contribution of the torsion angle by 84% to the activity of the inhibitors.

dipeptides / tripeptides / α-ketoamides / HIV-1 protease / inhibitory activity / QSAR

1. Introduction

A number of human immunodeficiency virus-1 protease (HIV-1 PR) inhibitors have been designed on the concept of a transition state mimic. One of these strategies was the insertion of hydroxymethylcarbonyl (HMC) core of allophenyl norstatine [Apns, (2*S*, 3*S*)-3-ainino-2-liydroxy-4-phenylbutyric acid] as a nonhydrolyzable isostere with the tetrahedral geometry at the Phe-Pro scissile amide bond. This design led to synthesis of many HIV-1 protease inhibitors containing Apns [1–10]. Among them the tripeptide KNI-272 (*figure 1*) is a highly selective and superpotent HIV-1 protease inhibitor ($K_i = 5.5$ PM) currently in clinical study [11–15]. Also KNI-241 (*figure 1*) showed strong inhibition against both wild-type HIV-protease and synthetic HIV-protease analogues [14, 15]. In addition, several small size dipeptides containing HMC core of Apns, e.g. KNI-413, KNI-549, KNI-727, KNI-577 and KNI-764 were recently synthesized and exhibited excellent HIV-protease inhibitory activity and were characterized by low toxicity and good pharmacokinetic properties [14, 15].

Oxidation of the hydroxyl group of the HMC in Phe-Pro resulted in the formation of an α-ketoamide core. α-Ketoamide isosteres have been successively used in the design of inhibitors for metalloprotein (like calcium activated cysteine protease, calpains) [16] and as a promising new core for the synthesis of HIV-1 protease inhibitors [17, 18]. By analogy α-ketoamides were prepared by oxidation of the HMC group in the norstatine-based HIV-1 protease inhibitors. The activity of the resultant α-ketoamides

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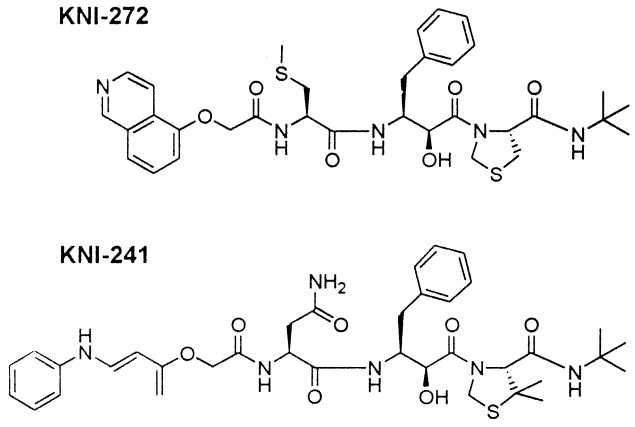


Figure 1.

was either comparable with or improved upon the corresponding HMC containing inhibitors [19–21]. The evident improvement of the activity of the α-ketoamides over the HMC precursors was found to be due to the hydration of the ketone carbonyl of the α-ketoamides to form *gem*-diol at the level of the active site of the protease, as shown by NMR [22]. These results prompted us to synthesize a new series of tripeptides incorporating Apns as a transition state mimic, containing α-ketoamide at P1 site (**18**–**21**) and to evaluate their activity as HIV-1 protease inhibitors. Other α-ketoamides were also prepared at P2 site (**8**–**12**) in the truncated dipeptide derivatives and their activity was also tested. This study was carried out to investigate the contribution of the α-ketoamide core in the norstatine-based HIV-1 protease inhibitors to their activity.

2. Chemistry

The preparation of the target peptide inhibitors necessitate the synthesis of the transition state element, allophenylnorstatine, Apns [3, 23–25]. The most simple diastereoselective method for the preparation of *N*-*tert*-butyloxycarbonyl (Boc)-Apns (*figure 2*) was that analogous to the preparation of allocyclohexylnorstatine [26]. Boc-L-phenylalaninol (**1**) [27, 28] was converted to the corresponding aldehyde, Boc-phenylalaninal (**2**), by controlled oxidation with sulfur trioxide. The aldehyde was hydrocyanated and hydrolyzed to provide 3-amino-2-hydroxy-4-phenylbutyric acid (AHPBA) as HCl salt (**3**). N-Protection of AHPBA by ditert-butyl pyrocarbonate $(Boc)_2O$,

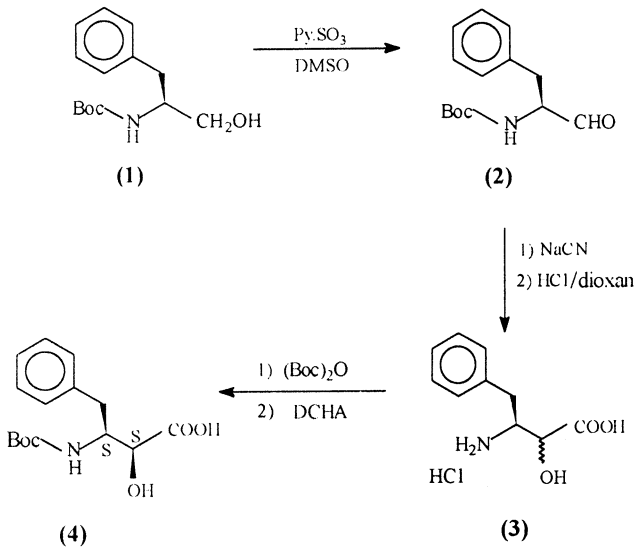


Figure 2.

provided 3:2 diastereomeric mixture of Boc-Apns (2*S*,3*S*) and Boc-Pns (2*R*,3*S*), respectively, as monitored by HPLC [9, 28]. The pure Boc-Apns (**4**) was exclusively crystallized out from ether as dicyclohexylamine (DCHA) salt. The purity of Boc-Apns ($\geq$ 98%) was checked by HPLC and ^{1}H-NMR. Its ^{1}H-NMR spectrogram was superimposable on the spectrogram of a reference sample.

The synthesis of the target dipeptides (*figure 3*) was started by amidation of Boc thiazolidine carboxylic acid (Boc-Thz-OH, **5**) with *tert*-butylamine followed by unmasking of the protected amino group to yield **6** which was coupled with C-activated Boc-Apns. Apns-Thz-NHBu^t (**7**) was reacted with the appropriate α-ketoacid to give the required compounds (**8**–**12**). The dipeptide isostere, Val-Apns-Thz-NHBu^t (**13**) was prepared by coupling of Apns-Thz-NHBu^t (**7**) with C-activated Boc-Valine followed by N-deprotection (*figure 3*). The target tripeptides (**18**–**21**) were obtained (*figure 4*) by oxidation of the transition state element, Apns of the previously synthesised, 3-{3(*S*)-[*N*-(5-isoquinolinyloxyacetyl)-L-valinyl]-amino-2(*S*)-hydroxy-4-phenylbutanoyl}-*N*(*tert*-butyl)-(un)substituted thiazolidine (oxazolidine)-4(*R*)-carboxamide, IQOA-Val-Apns-[(un)substituted-Thz(Oxa)]NHBu^t, (**14**–**17**) [29] to the corresponding α-ketoamides using dimethyl sulfoxide and dicyclohexylcarbodiimide (DMSO/DCC) as reported for similar compounds [17].

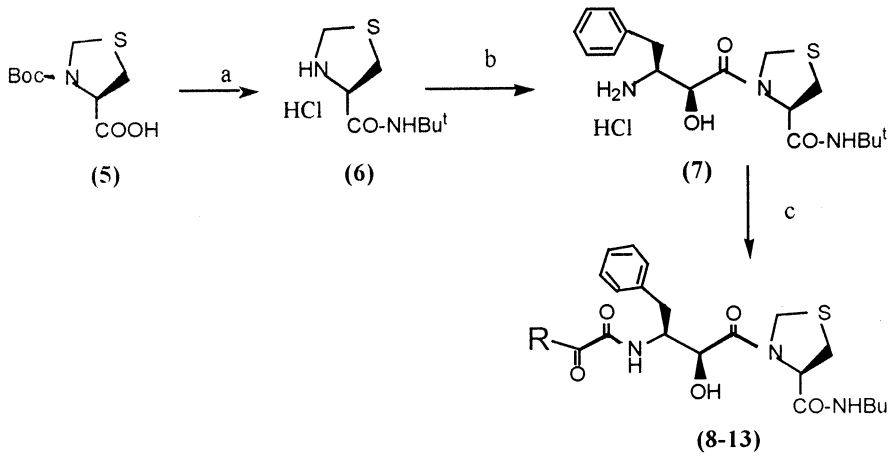


Figure 3. Reagents: a) i – DCC, HOBt, NH_2-Bu^t; ii – 4N HCl dioxane. b) i – EDC·HCl, HOBt, Et_3N·Boc-Apns; ii – 4N HCl dioxane; c) i – EDC·HCl, Hobt, Et_3N, Valine or α-keto acid.

3. Results and discussion

3.1. In vitro HIV-1 protease inhibitory activity

As a primary screen, di- and tripeptide analogues containing α-ketoamides as a new structural core were initially evaluated in vitro for inhibitory activity against HIV-1 protease according to reported technique [8]. Tripeptides containing an HMC core in P1 site were found to inhibit HIV-1 protease activity and mimic the Phe-Pro transition state in HIV-1 protease substrate [29]. In our research to improve the HIV-1 protease inhibitory activity, the hydroxymethyl group of the most active tripeptide derivatives, (**14**–**17**) was oxidized to α-keto analogues. The oxidized compounds **18**–**20** were about 5–10% more active than the corresponding hydroxymethyl containing compounds **14**–**16**, respectively. While oxidation of the hydroxymethyl group in compound **17** resulted in an equipment α-ketoamide **21** (*table I*).

It was found that, the ketone carbonyl of the α-ketoamide of certain peptides hydrated and formed *gem*-diol at the level of the active site of protease, as indicated by NMR studies in aqueous DMSO solution [22]. The observed improvement of the activity of the target α-ketoamides over the HMC precursors suggests that at the active site the formed *gem*-diol most probably enhances hydrogen bonding potentialities, which places the inhibitor in a more proper manner to be recognized by the enzyme.

The encouraging results of the α-ketoamides already tested at P1 residue in the tripeptides (**18**–**21**) suggested a route to the rational design of a low molecular weight peptidomimitics lacking P3 ligand and the resultant dipeptides (**8**–**12**) were tested at μM level of inhibitor concentration. In this series the α-ketoacid residues imparted a significant enhancement of protease inhibition activity (*table II*) as shown by the 4-fold improvement

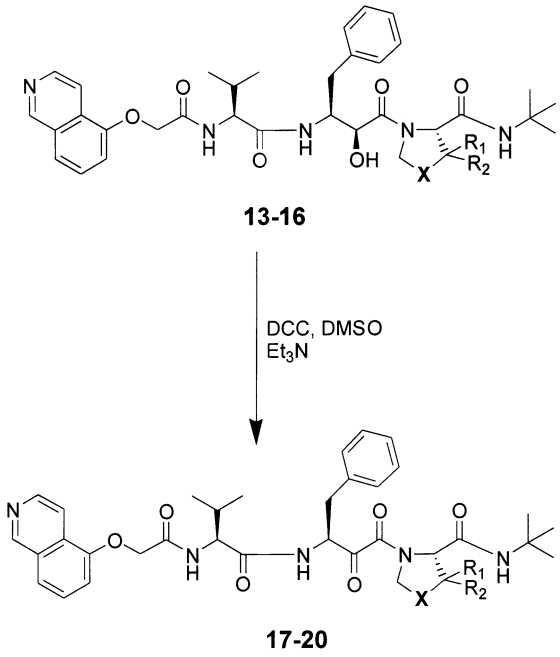
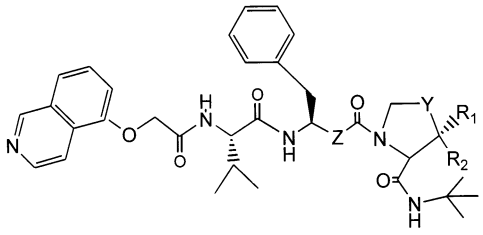


Figure 4.

Table I. HIV PR activity (%) of tripeptide (**14**–**17**) and their α-ketoamide analogues (**18**–**21**) at 50 nM concentration.



Compd. no.	Y	Z	R_1	R_2	Activity (%)
14	O	CHOH (S)	H	H	71.3
18	O	CO	H	H	76.3
15	S	CHOH (S)	H	H	88.5
19	S	CO	H	H	95.5
16	O	CHOH (S)	CH_3	H	86.3
20	O	CO	CH_3	H	96.4
17	S	CHOH (S)	CH_3	CH_3	97.7
21	S	CO	CH_3	CH_3	97.7

of activity of **9** over its isostere, Val-Apns-Thz-NHBu^t **13**. This observation is consistent with that already signalled in the tripeptide derivatives (**18**–**21**). As seen from *table II*, the activity gain goes in the order α-oxopropionoyl < α-oxopentanoyl and isopentanoyl < α-oxo-β-methylpentanoyl < α-oxoglutaroyl.

It is clear that elongation and branching of the chain might increase the activity of **8** by 10–20%, however a polar residue at the end of the chain doubled the enzyme inhibition activity (compound **12**).

3.2. Quantitative structure activity relationship of the dipeptides

In this series of dipeptide-based HIV-1 protease inhibitors several factors may affect their activity. Among these factors is the possible rotation of the α-keto group about the C^α bond. The angle of rotation can be simulated in a quantitative manner by the calculated torsion angle (Tor) of the linking bond C^α–C at Z moiety. A semi-empirical calculation program (MMX) was used for predicting the values of the torsion angle in compounds **8**–**12** (*table II*). Linear free energy (LFE) approach was used as a statistical model to derive the relation between HIV-1 protease inhibition activity and the values (Tor), Eq. (1).

HIV-1 protease inhibition activity

$$= 3.689\ (\pm 0.903)\ \text{Tor} - 7.253 \qquad (1)$$

$$n = 5,\ r = 0.921,\ \text{SE} = 5.106,\ P < 0.027$$

where n is the compound **8**–**12**; r is the linear regression coefficient and SE is the standard error at the confidence level $P < 0.05$.

Eq. (1) reveals that, in the given series of compounds the torsion angle C^α–C at Z moiety contributed by 84% to the inhibition activity. Other factors like branching at C^β may also be of significant effect.

4. Conclusion

The α-ketoamide isostere was a good transition-state mimetic in HIV-1 protease inhibitor design. Di- and tripeptides containing α-ketoamide at P2 or P1 site, respectively, exhibited a significant enhanced inhibitory activity relative to their parent isosteres and are shown to be a promising core structure for inhibition of HIV-1 protease for the development of more potent derivatives. The study suggested that the potency of the truncated dipeptide derivatives are greatly affected by the nature of' the incorporated α-ketoamide. QSAR study using LFE indicated that, the torsion angle at the α-diketone contributed by 84% to the activity in the dipeptide derivatives.

5. Experimental protocols

Allophenyl norstatine (reference sample) was obtained from Nippon Mining Co., Japan. Chemicals were of analytical grade and the solvents were dried (if necessary) over molecular sieve 4. All coupling and deprotection reactions were carried out under anhydrous conditions using an anhydrous silica drying tube.

The standard workup procedures were applied as follows, the reaction mixture was partitioned between the indicated solvent and water, the combined organic extracts were washed with suitable aqueous systems, 5% sodium bicarbonate (twice), 5% citric acid (twice), water and brine, then dried over anhydrous sodium sulphate, filtered, and evaporated in vacuo.

Melting points were determined on a micro hot plate of Yanagimoto micro melting point apparatus, using micro cover glass thickness No. 1, 0.12–0.17 mm and are uncorrected.

The optical rotations were measured on a union automatic digital polarimeter PM-101. The purity of the compounds was examined by TLC and HPLC.

Thin layer chromatography (TLC) was performed on precoated Merck silica gel sheets (0.25 mm) 60 F254, and the spots were visualized by UV and/or by ninhydrin reagent. The following solvent systems were used: (a) chloroform–methanol 40:1 (b) chloroform–methanol 20:1, or (c) hexane–ethyl acetate 2:1 for the N-protected compounds; (d) chloroform–methanol–water (lower layer) 8:3:1 system for the amine hydrochloride salts and (e) chloroform–methanol 10:1 system in the case of final compounds.

Column chromatography was carried out on Merck silica gel 60 (particle size 0.063–0.200 mm).

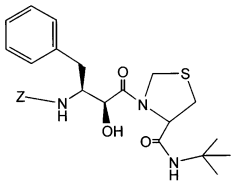
Preparative reversed-phase HPLC (Shimadzu Liquid chromatograph LC-4A, spectrophotometer detector SPD-2AS) was performed utilizing a YMC packed ODS-AM AM 12S05-2520 WT type SH-343-5AM column (250 × 20 mm i.d, S-5 120A) with gradient elution using acetonitrile in 0.1% aqueous trifluoroacetic acid solvent system. Reversed-phase HPLC was performed on a Hitachi 655 A-11 utilizing YMC packed ODS-AM AM-302 column (150 × 4.0 mm i.d S5 120A) thermostated at 38 °C. A gradient elution sequence with acetonitrile from 20 to 80% v/v (through 30 min) in 0.1% aqueous TFA was used at flow rate 0.7 mL/min. Spectrophotometeric detection at 230 nm was used and the retention time (Rt) recorded in minutes.

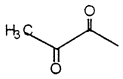
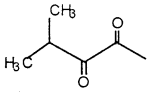
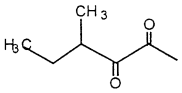
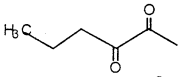
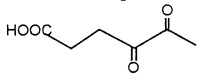
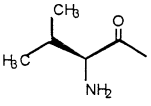
^{1}H-NMR was recorded on a JEOL JNM-EX 270 MHz spectrometer, all chemical shifts are given in (δ ppm) relative to tetramethylsilane (TMS) as internal standard.

FAB mass spectra (MS) and high resolution FAB MS (HRMS) were recorded as $(M + H)^+$ on a JEOL JMS-SX 102 AQQ hybrid mass spectometer using glycerol, thioglycerol, or magic bullet as internal reference. All the new compounds, obtained as lyophilized materials, gave satisfactory high resolution mass spectra (error less than 3 ppm) and were fully characterized spectroscopically.

Inhibition of HIV-1 protease activity was quantified by using a peptidolytic assay with a synthetic nona-peptide substrate Ac-Arg-Ala-Ser-Gln-Asn-Tyr-Pro-Val-Val-NH_2. The inhibitors were dissolved in DMSO and assayed at 50 nM and 5 μM concentrations of the target tripeptides and dipep-

Table II. Torsion angle of dipeptides (**8**–**13**) and their HIV-1 protease inhibitory activity



Compd No.	Z Group	Torsion angle[a]	Activity[b]
8		10.35	34.1
9		15.24	43.5
10		14.8	54.7
11		16.28	43.4
12		17.94	63.3
13		15.82	10.7

[a]Torsion angle at the α–diketone; [b] % of HIV-1 protease inhibition at 5 μm concentration of the inhibito

tides, respectively. The assay was performed at Bioscience Research Laboratories, Nippon Mining Co., Japan.

5.1. General method for N-protection and N-deprotection of amino acids

Synthesis of Boc-amino acids (N-protection) and removal of the Boc group (N-deprotection) were carried out according to reported methods [30–32].

5.2. (2S,3S)3-(tert-Butyloxycarbonyl)amino-2-hydroxy-4-phenyl-butanoic acid (Boc-Apns-OH) (**4**)

To a stirred solution of Boc-Phe-ol (**2**) [27, 28] (22.5 g, 89.6 mmol) in DMSO (31.4 mL), benzene (31.4 mL) and TEA (24.75 mL, 224 mmol) was added, portionwise, at − 15 °C, pyridine sulfur trioxide (28.57 g, 244 mmol) for 30 min. The reaction mixture was quenched with ice-water, extracted with EtOAc and the organic layer washed gently with water. Solution of potassium cyanide (13.15 g, 268.8 mmol) in a least amount of water (about 10 mL) was added to the EtOAc extract and the stirring continued overnight. The organic layer was separated, washed with water, brine, and evaporated under vacuum. To the residue, a mixture of conc. HCl and dioxane (60 mL, 1:1) was added and the reaction mixture was refluxed for 8 h, cooled and washed twice with ether. The aqueous layer was concentrated under vacuum at a temperature not exceeding 40 °C and to the residue in water (35 mL), acetone (200 mL) was added. The solution was cooled in an ice-bath, neutralized with 4 N NaOH to pH 5.5, kept overnight in a refrigerator and filtered to afford 6.45 g (36.9%) of (3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid (AHPBA-OH) with m.p. 246–247 °C.

A solution of AHPBA-OH (6.5 g, 333 mmol) in a mixture of THF (10 mL), water (30 mL) and TEA (6.94 mL, 50 mmol) was stirred and cooled in an ice-water bath. Solution of $(Boc)_2O$ (8 g, 36.63 mmol) in THF (7 mL) was added and the reaction proceeded as reported for preparation of Boc amino acid [31]. The resulting residue of Boc-AHPBA-OH diastereomeric mixture was dissolved in ether, dicyclohexylamine (DCHA) was added dropwise until pH 7–8. The solution was stirred by glass rod, kept for 1 day in refrigerator, and filtered. The residue was dissolved in water, covered with a layer of EtOAc (40 mL) and acidified with 10% solution of citric acid to pH 2–3. The aqueous phase was worked up (EtOAc, water, and brine) and yielded 3.2 g (32.5%) of Boc-Apns-OH (**4**) as white crystals, m.p. 145–147 °C as reported [24], $[\alpha]_D^{25}$ 0.0° ($c = 1.3$, MeOH). ^{1}H-NMR (DMSO-d_6) δ: 1.27, (s, 9H), 2.64 (d, 2H, $J = 8.3$ Hz), 3.37 (bs, 1H), 3.94–3.98 (m, 1H), 4.11 (d, 1H, $J = 5.8$ Hz), 6.72 (d, 1H, $J = 8.5$ Hz), 7.12–7.25 (m, 5H), 12.31 (s, 1H).

5.3. General coupling procedure

Using 1-ethyl-3-(3-dimethylaminopropyl)carbodimide hydrochloride, EDC·HCl, as reagent and 1-hydroxybenzotriazole monohydrate, $HOBt \cdot H_2O$ as coupling additive [33, 34].

5.3.1. 3-[3(S)-(Substituted)amino-2(S)-hydroxy-4-phenyl-butanoyl]-N(tert-butyl)-thiazolidine-4(R)-carboxamide, N-(Substituted)-Apns-Thz-NHBu^t (**8**–**13**)

The following compounds were prepared as described under the general procedure for coupling using Apns-Thz-NHBut (**7**) and the corresponding α-keto carboxylic acid or the amino acid, valine.

5.3.2. N-(2-Oxopropionyl)-Apns-Thz-NHBu^t (**8**)

Yield 81%, Rt = 18.6, m.p. 109–111 °C, $[\alpha]_D^{25}$ − 157.0° ($c = 0.50$, MeOH). ^{1}H-NMR (DMSO-d_6) δ: 1.25 (s, 9H), 2.24 (s, 3H), 2.79 (d, 2H, $J = 9.5$ Hz), 2.99 (dd, 1H, $J = 6.6$, 5.0 Hz), 3.30 (dd, 1H, $J = 7.6$, 5.0 Hz), 4.20–4.27 (m, 2H), 4.45 (s, 1H), 4.74 (d, 1H, $J = 9.1$ Hz), 4.79 (t, 1H, $J = 6.6$ Hz), 4.95 (d, 1H, $J = 9.2$ Hz), 7.15–7.26 (m, 5H), 7.65 (s, 1H), 8.41 (d, 1H, $J = 9.2$ Hz). MS (m/z): 436 $(M + H)^+$, HRMS 436.2202 (calcd. 436.1906), elemental composition $C_{21}H_{30}N_3O_5S$.

5.3.3. N-(3-Methyl-2-oxobutanoyl)-Apns-Thz-NHBu^t (**9**)

Yield 89%, Rt = 22.93, m.p. 131–133°C, $[\alpha]_D^{25}$ −131.1° ($c = 0.45$, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.97 (d, 3H, $J = 7.0$ Hz), 1.02 (d, 3H, $J = 7.0$ Hz), 1.32 (s, 9H), 2.00–2.18 (m, 1H), 2.75 (d, 2H, $J = 8.5$ Hz), 3.13 (dd, 1H, $J = 6.6$, 4.5 Hz), 3.33 (dd, 1H, $J = 7.0$ Hz), 4.19–4.27 (m, 2H), 4,46 (bs, 1H), 4.69 (d, 1H, $J = 9,5$ Hz), 4.79 (t, 1H, $J = 7.2$ Hz), 4.96 (d, 1H, $J = 9.2$ Hz), 7.15–7.26 (m, 5H), 7.67 (s, 1H), 8.42 (d, 1H, $J = 8.8$ Hz). MS (m/z): 464 $(M + H)^+$, HRMS 464.1902 (calcd. 464.1882), elemental composition $C_{23}H_{34}N_3O_5S$.

5.3.4. N-(2-Oxopentanoyl)-Apns-Thz-NHBu^t (**10**)

Yield 90%, Rt = 25.34, m.p. 119–120°C, $[\alpha]_D^{25}$ − 115.8° ($c = 0.50$, MeOH). ^{1}H-NMR (DMSO-d_6) δ: 0.82 (t, 3H, $J = 7.3$ Hz), 0.94–1.07 (m, 2H) 1.25 (s, 9H), 1.43 (t, 2H, 7.3 Hz), 2.79 (d, 2H, $J = 9.6$ Hz) 2.98 (dd, 1H, $J =$ 6.6, 5.3 Hz), 3.30 (dd, 1H, $J = 7.6$, 4.0 Hz), 4.13–

4.20 (m, 2H), 4.44 (s, 1H), 4.55 (d, 1H, $J = 8.9$ Hz), 4.75 (t, 1H, $J = 7.0$ Hz), 4.96 (d, 1H, $J = 8.9$ Hz), 7.13–7.29 (m, 5H), 7.60 (s, 1H), 8.41 (d, 1H, $J = 8.9$ Hz). MS (m/z): 464 $(M + H)^+$, HRMS 464.1899 (calcd. 464.1882), elemental composition $C_{23}H_{34}N_3O_5S$.

*5.3.5. N-(3-Methyl-2-oxopentanoyl)-Apns-Thz-NHBu^t (**11**)*

Yield 80%, Rt = 27.30, m.p. 121–124°C, $[\alpha]_D^{25}$ −69.5° ($c = 0.50$, MeOH). ^{1}H-NMR (DMSO-d_6) δ: 0.65 (t, 3H, $J = 8.1$ Hz), 0.74–0.81 (m, 2H), 0.91 (d, 3H, $J = 6.9$ Hz), 1.25 (s, 9H), 1.50–1.57 (m, 1H), 2.76 (d, 2H, $J = 10.0$ Hz), 2.99 (dd, 1H, $J = 6.6$, 5.0 Hz), 3.03–3.11 (m, 1H), 4.15–4.22 (m, 2H), 4.45 (bs, 1H), 4.59 (d, 1H, $J = 8.9$ Hz), 4.76 (t, 1H, $J = 7.0$ Hz), 4.97 (d, 1H, $J = 9.2$ Hz), 7.12–7.26 (m, 5H), 7.61 (s, 1H), 8.48 (d, 1H, $J = 8.9$ Hz). MS (m/z): 478 $(M + H)^+$, HRMS 478.2303 (calcd. 478.2301), elemental composition $C_{24}H_{36}N_3O_5$.

*5.3.6. N-(4-Carboxy-2-oxobutanoyl)-Apns-Thz-NHBu^t (**12**)*

Yield 90%, Rt = 16.36, m.p. 114–117°C, $[\alpha]_D^{25}$ −135.0° ($c = 0.50$, MeOH). ^{1}H-NMR ($CDC1_3$) δ: 1.31 (s, 9H), 2.72 (d, 2H, $J = 8.5$ Hz), 2.79 (t, 2H, $J = 6.3$ Hz), 2.98 (t, 2H, $J = 5.8$ Hz), 3.12 (dd, 1H, $J = 6.5$, 5.5 Hz), 3.43 (dd, 1H, $J = 6.9$, 5.7 Hz), 4.29–4.37 (m, 2H), 4.48 (s, 1H), 4.69 (d, 1H, $J = 9.2$ Hz), 4.79 (t, 1H, $J = 7.1$ Hz), 5.00 (d, 1H, $J = 9.3$ Hz), 7.18–7.27 (m, 5H), 7.68 (s, 1H), 8.42 (d, 1H, $J = 7.9$ Hz), 12.03 (bs, 1H). MS (m/z): 494 $(M + H)^+$, HRMS 494.1871 (calcd. 494.1882), elemental composition $C_{23}H_{32}N_3O_7S$.

*5.3.7. Val-Apns-Thz-NHBu^t·HC1 (**13**)*

Yield 76%, m.p. 153–166°C, $[\alpha]_D^{25}$ −118.0° ($c = 1.0$, MeOH). ^{1}H-NMR (DMSO-d_6) δ: 0.76 (dd, 6H, $J = 6.4$, 11.5 Hz), 1.27 (s, 9H), 1.93–2.01 (m, 1H), 2.75 (d, 2H, $J = 7.8$ Hz), 3.0 (dd, 1H, $J = 5.5$, 4.2 Hz), 3.36 (dd, 1H, $J = 5.5$, 5.0 Hz), 3.72–3.80 (h, 2H), 4.31–4.36 (m, 3H), 4.43 (bs, 1H), 4.66 (d, 1H, $J = 7.6$ Hz), 4.79 (t, 1H, $J = 6.9$ Hz), 4.95 (d, 1H, $J = 8.2$ Hz), 7.09–7.20 (m, 5H) and 8.24 (d, 1H, $J = 6.9$ Hz).

*5.3.8. {3(S)-[N-(5-Isoquinolinyloxyacetyl)-L-valinyl]-amino-2-oxo-4-phenyl-butanoyl}-N(tert-butyl)-(un)-substituted thiazolidine (oxazolidine)-4(R)-carboxamide IQOA-Val-Apns(C=O)-((un)substituted-Thz(Oxa))-NHBu^t, (**18**–**21**) General procedure for oxidation of Apns in tripeptides (**14**–**17**) [17]*

Trifluoroacetic acid (4.0 μL, 0.054 mmol) and the appropriate tripeptide (0.108 mmol) were added to a mixture of DCC (66.4 mg, 0.332 mmol), pyridine (8.0 μL, 0.108 mmol), and DMSO (320 μL) in benzene (700 μL) at 0°C. The mixture was stirred for 22 h at room temperature, then EtOAc (1 mL) was added. The resulting insoluble substance was filtered off. The filtrate was worked up (EtOAc, $NaHCO_3$) and purified by preparative HPLC to give the corresponding α-ketoamide as a white powder.

*5.3.9. iQOA-Val-Apns(C=O)-Oxa-NHBu^t (**18**)*

The titled compound was obtained by oxidation of iQOA-Val-Apns-Oxa-NHBut (**14**): Yield 34%, m.p. 142–144°C, $[\alpha]_D^{25}$ −120.3° ($c = 0.32$, MeOH), Rt = 14.63. ^{1}H-NMR (DMSO-d_6) δ: 0.83 (dd, 6H, $J = 6.6$ Hz), 1.28 (s, 9H), 1.90–2.03 (m, 1H), 2.83 (d, 2H, $J = 8.5$ Hz), 3.42 (dd, 1H, $J = 6.6$, 4.9 Hz), 3.72 (dd, 1H, $J = 7.5$, 3.8 Hz), 4.22 (dd, 1H, $J = 7.0$ Hz), 4.35–4.41 (m, 1H), 4.80 (t, 1H, $J = 6.7$ Hz), 4.85 (s, 2H), 5.06 (d, 1H, $J = 8.3$ Hz), 5.11 (d, 1H, $J = 8.5$ Hz), 7.07 (d, 1H, $J = 7.2$ Hz), 7.16–7.32 (m, 6H), 7.73 (s, 1H), 7.79 (d, 1H, $J = 7.0$ Hz), 7.89 (t, 1H, $J = 6.1$ Hz), 7.92 (d, 1H, $J = 8.6$ Hz), 8.48 (d, 1H, $J = 7.0$ Hz), 8.59 (d, 1H, $J = 7.0$ Hz), 9.65 (s, 1H). MS (m/z): 632 $(M + H)^+$, HRMS 632.3082 (calcd. 632.3084) elemental composition $C_{34}H_{42}N_5O_7$.

*5.3.10. iQOA-Val-Apns(C=O)-Thz-NHBu^t (**19**)*

The titled compound was obtained by oxidation of iQOA-Val-Apns-Thz-NHBut (**15**). Yield 40%, m.p. 135–137°C, $[\alpha]_D^{25}$ 120.5° ($c = 0.50$, MeOH), Rt = 18.41. ^{1}H-NMR (DMSO-d_6) δ: 0.82 (dd, 6H, $J = 7.0$ Hz), 1.27 (s, 9H), 1.92–2.03 (m, 1H), 2.84 (d, 2H, $J = 8.6$ Hz), 3.02 (dd, 1H, $J = 6.6$, 5.6 Hz), 3.42 (dd, 1H, $J = 7.5$, 4.2 Hz), 4.33 (dd, 1H, $J = 6.8$ Hz), 4.48–4.54 (m, 1H), 4.70 (t, 1H, $J = 8.2$ Hz), 4.83 (s, 2H), 4.88 (d, 1H, $J = 8.1$ Hz), 5.02 (d, 1H, $J = 8.6$ Hz), 7.15–7.24 (m, 6H), 7.64 (d, 1H, $J = 6.8$ Hz), 7.67 (s, 1H), 7.79 (d, 1H, $J = 7.9$ Hz), 7.87 (t, 1H, $J = 6.3$ Hz), 8.13 (d, 1H, $J = 6.6$ Hz), 8.58 (d, 1H, $J = 6.7$ Hz), 8.67 (d, 1H, $J = 7.3$ Hz), 9.43 (s, 1H). MS (m/z): 648 $(M + H)^+$, HRMS 648.2847 (calcd. 648.2855) elemental composition $C_{34}H_{42}N_5O_6S$.

*5.3.11. iQOA-Val-Apns(C=O)-[5-(R)-methyl-Oxa]-NHBu^t (**20**)*

The titled compound was obtained by oxidation of iQOA-Val-Apns-[5-(*R*)-methyl-Oxa-NHBu^t (**16**): Yield 55.9%, Rt = 16.37, m.p. 142–143°C, $[\alpha]_D^{25}$ −23.50° ($c = 0.50$, MeOH), ^{1}H-NMR ($CDCl_3$) δ: 0.82 (dd, 6H, $J = 6.7$ Hz), 1.32 (s, 9H), 1.47 (d, 3H, $J = 6.4$ Hz), 1.95–2.06 (m, 1H), 2.81 (d, 2H, $J = 8.6$ Hz), 3.36–3.42 (m, 1H),

4.22 (dd, 1H, $J = 7.0$ Hz), 4.31 (t, 1H, $J = 6.5$ Hz), 4.81 (d, 1H, $J = 6.7$ Hz), 4.85 (s, 2H), 4.98 (d, 1H, $J = 6.3$ Hz), 5.08 (d, 1H, $J = 7.5$ Hz), 7.09 (d, 1H, $J = 7.2$ Hz), 7.16–7.32 (m, 6H), 7.73 (s, 1H), 7.79 (d, 1H, $J = 7.0$ Hz), 7.89 (t, 1H, $J = 6.1$ Hz), 7.92 (d, 1H, $J = 8.6$ Hz), 8.48 (d, 1H, $J = 7.0$ Hz), 8.59 (d, 1H, $J = 7.0$ Hz), 9.65 (s, 1H). MS (m/z): 646 $(M + H)^+$, HRMS 646.3242 (calcd. 646.3240) elemental composition $C_{35}H_{44}N_5O_7$.

*5.3.12. iQOA-Val-Apns(C=O)-(5,5-dimethyl-Thz)-NHBu^t (**21**)*

The titled compound was prepared by oxidation of iQOA-Val-Apns-(5,5-dimethyl-Thz)-NHBu^t (**17**): Yield 35%, m.p. 130–132°C, $[\alpha]_D^{25}$ −73.0° ($c = 0.45$, MeOH), Rt = 19.80. ^{1}H-NMR (DMSO-d_6) δ: 0.77 (dd, 6H, $J =$ 6.5 Hz), 1.29 (s, 9H), 1.40 (d, 3H, $J = 9.5$ Hz), 1.48 (d, 3H, $J = 9.5$ Hz), 1.93–1.98 (m, 1H), 2.76 (d, 2H, $J = 9.3$ Hz), 4.28 (dd, 1H, $J = 6.5$ Hz), 4.30–4.36 (m, 1H), 4.77 (s, 2H), 4.84 (s, 1H), 4.84 (d, 1H, $J = 7.7$ Hz), 5.00 (d, 1H, $J = 8.5$ Hz), 7.09–7.26 (m, 6H), 7.71 (s, 1H), 7.76 (t, 1H, $J = 6.5$ Hz), 7.97–8.09 (m, 2H), 8.27 (d, 1H, $J = 8.2$ Hz), 8.35 (d, 1H, $J = 9.8$ Hz), 8.60 (d, 1H, $J = 9.9$ Hz), 9.57 (s, 1H). MS (m/z): 676 $(M + H)^+$, HRMS 676.3164 (calcd. 676.3168) elemental composition $C_{36}H_{46}N_5O_6S$.

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