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Original article

Design, synthesis and biological evaluation of new peptide-based ureas and thioureas as potential antagonists of the thrombin receptor PAR1

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ABSTRACT

By applying a diversity oriented synthesis strategy for the search of new antagonists of the thrombin receptor PAR1, a series of peptide-based ureas and thioureas, including analogues of the PAR1 reference antagonist RWJ-58259, has been designed and synthesized. The general synthetic scheme involves reduction of basic amino acid-derived amino nitriles by hydrogen transfer from hydrazine monohydrate in the presence of Raney Ni, followed by reaction with diverse isocyanates and isothiocyanates, and protecting group removal. All new compounds have been evaluated as inhibitors of human platelet aggregation induced by the PAR1 agonist SFLLRN. Some protected peptide-based ureas displayed significant antagonist activity.

1. Introduction

In addition to the key role of thrombin in the blood coagulation cascade [1], this serine protease regulates multiple effects on an increasing variety of cells, such as: platelets [2,3], endothelial and smooth muscle cells [2,4], neurons and astrocytes in the nervous system [2,4–7], immune and inflammatory cells [8,9], osteoblasts [10], and tumor cells [11–14]. These cellular effects are mainly mediated by the activation of the protease-activated receptor PAR1 [15]. This is a G-protein coupled receptor (GPCR) that is activated by the thrombin-catalysed cleavage of the N-terminal extracellular domain at the Arg^{41}/Ser^{42} peptide bond, which unveils the recognition sequence SFLLRN that acts as a tethered activation ligand. The conformational changes induced by activation favours the coupling of PAR1 with heterotrimeric G-proteins, which consequently activate complex cellular signaling cascades [16,17].

Since PAR1 is mainly expressed in platelets, where its activation induces aggregation, it has been proposed that PAR1 antagonists could be good antithrombotic agents without the hemorrhagic drawbacks of thrombin inhibitors. Based on this suggestion, up to now, PAR1 antagonists have been searched almost exclusively in relation to the cardiovascular system [3,18,19]. However, numerous studies have shown that PAR1 is overexpressed in invasive and metastatic tumors and that its expression levels directly correlate with the degree of invasiveness of the cancer [20–28]. Based on these facts, this receptor is starting to be also considered a promising target for cancer therapy [15], particularly in the search of angiogenesis inhibitors [29].

The first potent PAR1 antagonists were SFLLRN-based peptidomimetic ureas, represented by the optimized antagonist RWJ-58259 (Fig. 1) [19]. This antagonist showed protection against thrombus formation in nonhuman primates, providing the first *in vivo* proof for supporting the potential clinical utility of PAR1 antagonists, although, its low oral bioavailability stopped its clinical development [19]. Later, several laboratories have reported a few series of antagonists obtained from HTS of diverse libraries of non-peptide small molecules, followed by optimization [19,30]. The most advanced of these antagonists is SCH-530348 (named vorapaxar, Fig. 1), derived from the natural product himbacine, which currently is undergoing Phase III clinical trials in patients with acute coronary syndrome and in patients with atherosclerosis [31–33]. Up to now, there is no structural information on the binding sites of these PAR1 antagonists to be used for structure-based design of new antagonists. However, recent mutagenesis studies

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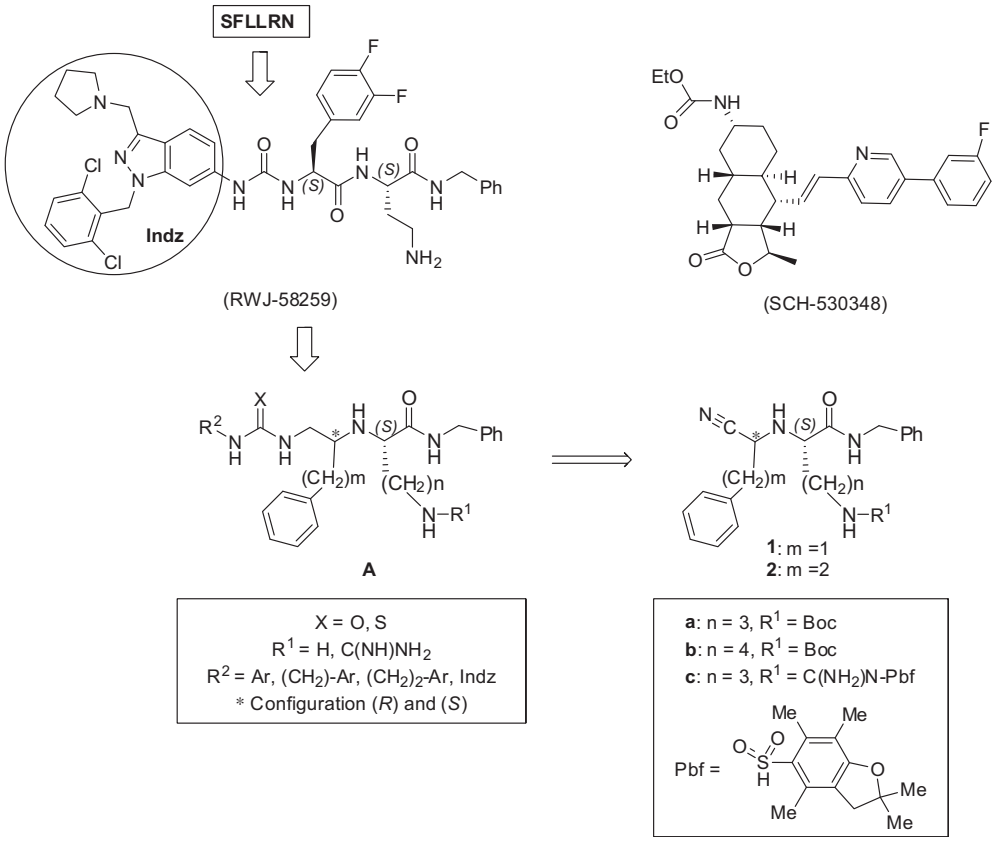


Fig. 1. Selected PAR1 antagonists and proposed new structures.

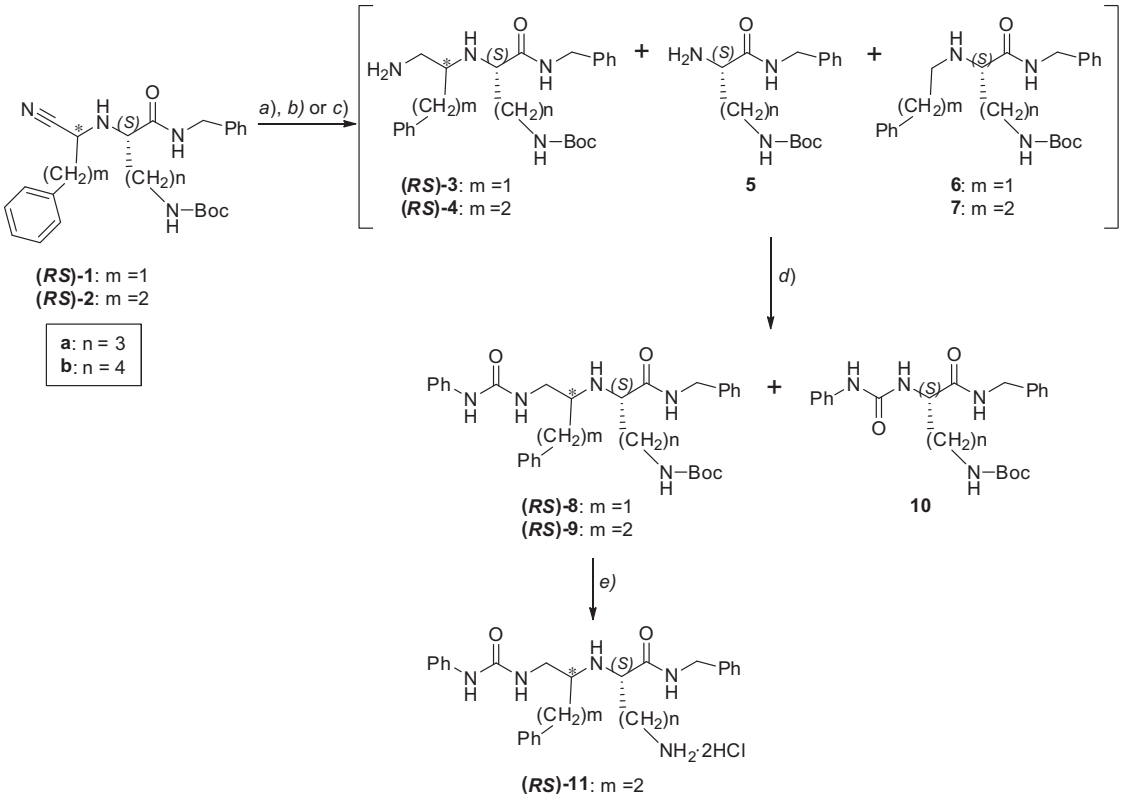
on thrombin and/or PAR1 [34–42], X-ray of thrombin crystallized with diverse N-terminal fragments of PAR1 [43,44], and NMR studies on the (Ala^{26}–Hse^{103}) N-terminal sequence of PAR1 [45] have shown that the first thrombin/PAR1 interaction is produced between the exosite I of thrombin and the hirudin-like sequence of PAR1 ($K^{51}YEPF^{55}$), and that this first interaction is essential and determinant for high affinity. It seems that the hydrophobic residues F34, I82, L65 and Y76, and the basic residues R67 and R73, of the exosite I of thrombin are important for high affinity. Taking into account this knowledge, we started a project directed to the search of new PAR1 antagonists based on these hot spots of the exosite I of thrombin for PAR1. As these hot spots are discontinuous and are not localized in a defined secondary structure, we decided to use a diversity oriented synthesis (DOS) strategy for the search of peptidomimetics. For this purpose, we planned the synthesis of diverse small directed libraries of different scaffolds able to display, at least, one or two aromatic groups and one or two basic groups at variable distances and orientations. Among these structures, our first objective was the synthesis of ureas and thioureas of general formula **A**, which could be considered as analogues of the reference antagonist RWJ-58259. To this aim, we devised a synthetic scheme using basic amino acid derived α-amino nitriles **1** and **2** (Fig. 1) as key diversity generation intermediates [46]. Herein, we report the synthesis of ureas and thioureas **A** and their evaluation as human PAR1 antagonists in a platelet aggregation assay.

2. Results and discussion

2.1. Synthesis

The proposed general synthetic scheme for the preparation of ureas and thioureas **A** involves cyano-reduction of the protected α-amino nitriles **1** and **2**, followed by reaction of the resulting primary amines with isocyanates or isothiocyanates and subsequent removal of protecting groups. The starting basic amino acid-derived α-amino nitriles **1** and **2** were obtained by a modified Strecker reaction as (1:1) epimeric mixtures at the cyano-supporting stereocenter, except for the arginine derivatives **1c**, which were obtained in an (*R*)/(*S*) ratio of (1:3) [47]. Only the epimeric mixtures (***RS***)-**1a**,**b** could be chromatographically resolved into the respective (*R*)- and (*S*)-epimers. Therefore, (***RS***)-**1c** and (***RS***)-**2a**,**b** were used as such epimeric mixtures through the synthetic pathway.

The epimeric mixture (***RS***)-**1a** was used as a model for optimizing the cyano reduction. Initially, this was attempted by 10% Pd(C) or Raney Ni catalysed hydrogenation. However, as shown in Scheme 1 and Table 1, the HPLC MS analysis of the crude reaction showed that the expected primary amines (***RS***)-**3a** were obtained along with variable percentages of **5a** and **6a**, resulting from retro-Strecker reaction and reduction of the intermediate imine after removal of HCN, respectively. The difficulties in the purification of (***RS***)-**3a** from the crude reaction mixtures moved us to try the reduction by hydrogen transfer, using hydrazine monohydrate as hydrogen source and Raney Ni as catalyst [48]. After the study of reaction conditions (Table 1), the formation of the side product **6a** could be completely avoided, but not that of the retro-Strecker reaction **5a**. This side reaction was minimized to a 5% by carrying out the reduction under refluxing MeOH for 5 min, using 20 equivalents of hydrazine monohydrate and 100 mg/mmol of Raney Ni (entry 7). These optimized conditions were applied to the reduction of the other starting α-amino nitriles. It is interesting to point out the importance of the Raney Ni activation, as well as the efficiency of the stirring, to make the reduction as faster as possible, in order to minimize the presence of the amino nitrile within the reaction medium, for minimizing the retro-Strecker reaction.



Scheme 1. *Reagents and conditions*: (a) H_2, Pd(C), MeOH, rt. (b) H_2, Raney Ni, MeOH, rt. (c) $NH_2NH_2 \cdot H_2O$, Raney Ni, MeOH or EtOH, *T* and time as indicated in Table 1. (d) PhNCO, CH_2Cl_2, 0 °C. (e) 3 M HCl, EtOAc, rt.

Besides, in the phenylacetaldehyde derivatives **3** ($m = 1$), the reduction of the (*R*)-epimer was slower than that of the corresponding (*S*)-epimer and, consequently, its percentage of degradation via retro-Strecker was significantly higher [see for example different yields for reduction of (*R*)- and (*S*) epimers of **1a** and **1b** in Table 2]. In the case of the Arg derivative **3c**, the difference in reactivity between epimers made that the minor (*R*)-epimer were lost in the reduction.

Due to the difficulties found in the separation of the primary amines **3** and **4** from the side product of the retro-Strecker reaction **5**, the crude reduction mixtures were initially used without purification for the synthesis of the desired ureas **8** and **9**. However, the difficulties in the purification of these ureas remained, and several column chromatographies were required for their separation from the ureas **10**, byproducts derived from the amino amides **5**, with considerable loss in the yield. Only the epimeric mixture of ornithine-derived ureas (***RS***)-**9a** could be resolved during this chromatographic purification. In view of these results, to avoid the purification difficulties, we decided to isolate the reduction intermediate amines as the corresponding Fmoc-derivatives. This strategy was studied in the phenylacetaldehyde derivatives (***R***)- and (***S***)-**3a**,**b** and (***RS***)-**3c**. At this point, it is important to note that when the Fmoc-protection was carried out under the standard basic conditions, using Na_2CO_3 as HCl acceptor in dioxane/water [49], almost complete epimerization of one of the two chiral centres was observed. This epimerization was completely avoided

Table 1
Optimization of the reduction conditions for (***RS***)-**1a**.

Entry	Catalyst	H_2 source	*T* (°C)	*t* (min)	Yield (%)[a]		
					(***RS***)-**3a**	**5a**	**6a**
1	10% Pd(C)	H_2	rt	24 h	80	8	12
2	Raney Ni (100 mg/mmol)	H_2	rt	24 h	54	6	40
3	Raney Ni (100 mg/mmol)	$NH_2NH_2 \cdot H_2O$	85	30	37	63	–
4	Raney Ni (100 mg/mmol)	$NH_2NH_2 \cdot H_2O$	rt	30	67	33	–
5	Raney Ni (200 mg/mmol)	$NH_2NH_2 \cdot H_2O$	rt	30	73	27	–
6	Raney Ni (100 mg/mmol)	$NH_2NH_2 \cdot H_2O$	rt	90	92	8	–
7	Raney Ni (100 mg/mmol)	$NH_2NH_2 \cdot H_2O$	65	5	95	5	–

[a] Determined by HPLC-MS analysis.

Table 2
Optimization of the synthesis of ureas **8**, **9** and **13**–**17**.

Amino nitrile	Diamine[a]		Urea[b]		
	N°	%	N°	A (%)[c]	B (%)[d]
(***RS***)-**1a** (1:1)[e]	(***RS***)-**3a** (1:3)[e]	74	(***RS***)-**8a** (1:3)[e]	36	52
(***R***)-**1a**	(***R***)-**3a**	55	(***R***)-**8a**	–	17
(***S***)-**1a**	(***S***)-**3a**	92	(***S***)-**8a**	–	67
(***RS***)-**1b** (1:1)[e]	(***RS***)-**3b** (1:1.3)[e]	73	(***RS***)-**8b** (1:1.3)[e]	21	44
(***R***)-**1b**	(***R***)-**3b**	60	(***R***)-**8b**	–	44
(***S***)-**1b**	(***S***)-**3b**	87	(***S***)-**8b**	–	57
(***RS***)-**1c** (1:3)[e]	(***S***)-**3c**	64	(***S***)-**8c**	–	25
(***R***)-**1a**	(***R***)-**3a**	55	(***R***)-**13a**	–	25
(***S***)-**1a**	(***S***)-**3a**	92	(***S***)-**13a**	–	51
(***R***)-**1b**	(***R***)-**3b**	60	(***R***)-**14b**	–	40
(***S***)-**1b**	(***S***)-**3b**	87	(***S***)-**14b**	–	52
(***R***)-**1b**	(***R***)-**3b**	60	(***R***)-**15b**	–	42
(***S***)-**1b**	(***S***)-**3b**	87	(***S***)-**15b**	–	61
(***R***)-**1b**	(***R***)-**3b**	60	(***R***)-**16b**	–	40
(***S***)-**1b**	(***S***)-**3b**	87	(***S***)-**16b**	–	62
(***RS***)-**2a** (1:1)[e]	(***RS***)-**4a** (1:1)[e]	74	(***RS***)-**9a** (1:1)[e]	27	–
(***RS***)-**2b** (1:1)[e]	(***RS***)-**4b** (1:1)[e]	74	(***RS***)-**9b** (1:1)[e]	29	–

[a] Yields determined by HPLC-MS analyses.
[b] Isolated yields.
[c] Overall yield of reduction, followed by reaction with the corresponding isocyanate (Method A).
[d] Overall yield of reduction, followed by sequential Fmoc-protection, isolation, Fmoc-deprotection, and reaction with the corresponding isocyanate (Method B).
[e] (*R*:*S*)-epimer ratio.

by using propylene oxide as HCl acceptor in CH_2Cl_2, to obtain the Fmoc-protected amines (***R***)- and (***S***)-**12a**,**b** and (***S***)-**12c** in 30–72% overall yield from the amino nitriles (***R***)- and (***S***)-**3a**,**b** and (***RS***)-**3c** (Scheme 2).

The Fmoc-removal in **12a–c**, followed by reaction with different aromatic isocyanates (Scheme 2) led to the desired ureas **8** and **13–16** in significantly higher overall yields than by the direct synthesis of the urea after the cyano-reduction (compare yields of methods A and B in Table 2).

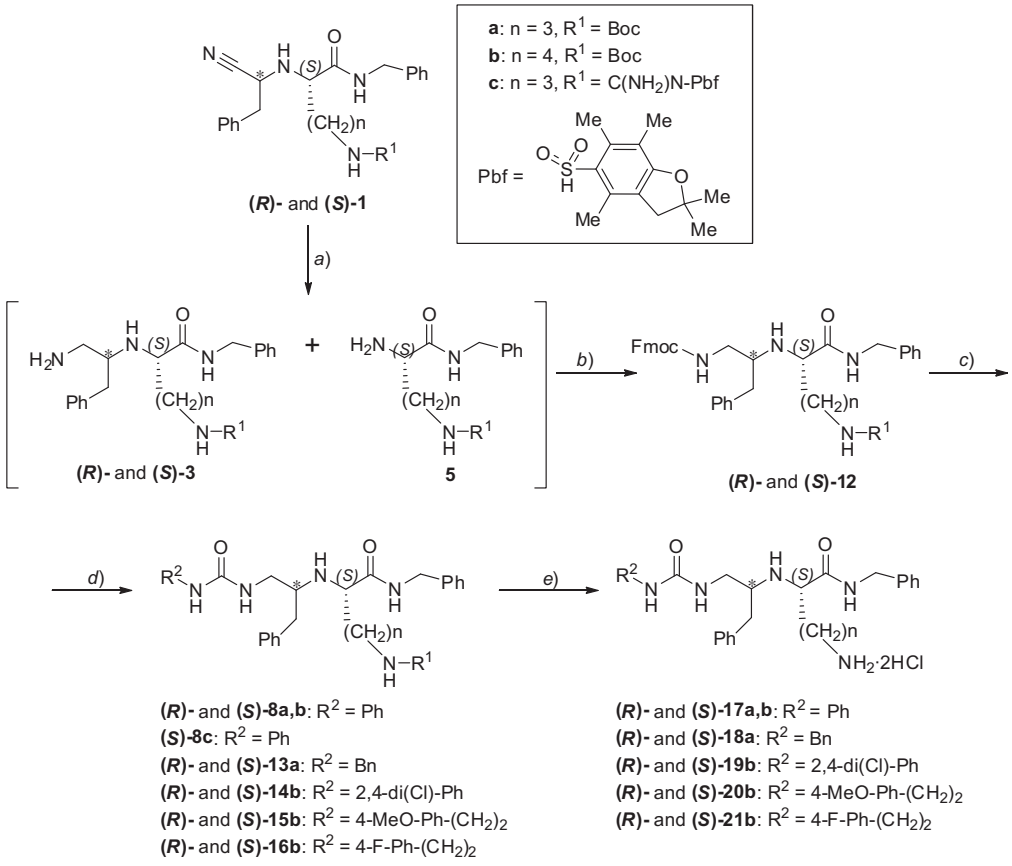
Removal of the *N*-Boc protection of the basic side chain in the Orn and Lys derived ureas **8a**,**b**, **9a**,**b** and **13a**,**b–16b** by treatment with 3 M HCl solution in EtOAc, yielded the corresponding deprotected compounds **11a**,**b** and **17a**,**b–21b** (Schemes 1 and 2). In view of the poor biological results of these deprotected ornithine and lysine derivatives (see below) and the low overall yield of the arginine-derived urea **8c**, this urea was not deprotected.

The lysine-derived Fmoc protected diamine **12b** was also used for the synthesis of the PAR1 reference antagonist RWJ-58259 analogue (***S***)-**25b** (Scheme 3). This urea was synthesized by applying our recently reported procedure for the preparation of RWJ-58259 [50]. This procedure involves the *in situ* formation of the indazole-derived isocyanate **23**, by reaction of the required 6-amino-indazole **22** with triphosgene in the presence of propylene oxide as HCl acceptor, followed by reaction with the deprotected diamines (***R***)- and (***S***)-**3b**. Interestingly, as commented for the reduction of the starting amino nitriles, the application of this methodology gave considerably higher yield for (***S***)-**24b** [85% overall from (***S***)-**12b**] than for its epimer (***R***)-**24b** [20% overall yield from (***R***)-**12b**]. Due to this low yield, this epimer was reserved for the biological evaluation and was not deprotected.

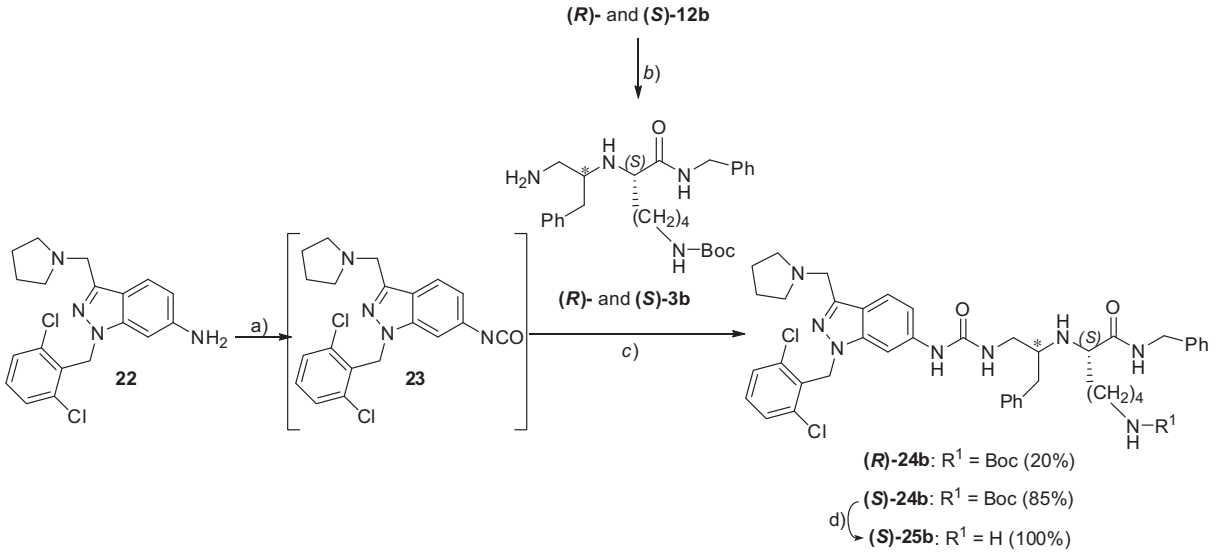
The optimized method B of synthesis of urea derivatives **17a** was similarly applied to the synthesis of the thiourea analogues (***R***)- and (***S***)-**27a**, by replacing the phenylisocyanate by phenyl isothiocyanate (Scheme 4).

2.2. Biological evaluation

Since PAR1 is mainly expressed in platelets, to evaluate the PAR1 antagonist activity, all new compounds were screened as inhibitors of human platelet aggregation induced by a 30 μM concentration of the PAR1 agonist SFLLRN. The antagonist RWJ-58259 was used as a reference. At a 10 μM concentration this antagonist inhibited 98% the platelet aggregation. All compounds were tested at an initial concentration of 0.1 mg/mL (≈150 μM). Unfortunately, none of the deprotected ureas or thioureas inhibited the platelet aggregation. However, as shown in Fig. 2, some of the protected ureas, and particularly the Fmoc intermediates **12**, showed significant inhibition. Thus, the Fmoc derivatives (***S***)-**12a** and (***S***)-**12c** inhibited a 51% and a 36%, respectively, the platelet aggregation. Among the protected ureas and thioureas, the most potent were the urea (***R***)-**8b** and the thiourea (***S***)-**26a**, which showed 38 and 33% of inhibition, respectively. Although these results do not allow to establish defined structure–activity relationships, it seems that in the Fmoc derivatives **12** and thioureas **26a**, the (*S*)-epimers showed higher inhibition % than the respective (*R*). However, in the case of the urea derivatives there is not a clear stereochemistry–activity relationship, as the results varied depending on the urea substitution. At this position, the phenyl group was the best (compare ureas **8b** with **14b–16b** and **24b**). Respecting the influence of the basic amino acid moiety, from the comparison of analogue (*S*)-epimers,



Scheme 2. *Reagents and conditions*: (a) $NH_2NH_2 \cdot H_2O$, Raney Ni, MeOH, 65 °C. (b) Fmoc-Cl, propylene oxide, CH_3CN, 0 °C. (c) Et_2NH, CH_2Cl_2, rt. (d) R^2NCO, CH_2Cl_2, 0 °C. (e) 3 M HCl, EtOAc, rt.



Scheme 3. *Reagents and conditions*: (a) $(CCl_3O)_2CO$, propylene oxide, THF, 0 °C. (b) Et_2NH, CH_2Cl_2, rt. (c) THF, 0 °C (d) 3 M HCl, EtOAc, rt.

in the Fmoc-derivatives **12**, the order of activity was Orn(**a**) > Arg(**c**) > Lys(**b**), while in the urea-derivatives **8**, the order was Arg(**c**) ≥ Lys(**b**) > Orn(**a**).

In the structural comparison of the inactive indazole-derived urea (***S***)-**25b** with the family of peptidomimetic urea PAR1 antagonists, to which the reference antagonist RWJ-58259 belongs [51], the main difference is the replacement of the di(F)-Phe residue of RWJ-58259 by the 1-amino-3-phenyl-propan-2-yl moiety in (***S***)-**25b** (see Fig. 1). This replacement involves the loss of the carbonyl group of the peptide bond, which could be important for the interaction with the PAR1 receptor, participating in H-bond formation or for the appropriate orientation of the pharmacophoric groups.

3. Conclusion

A series of peptide-based ureas and thioureas, including analogues of the PAR1 reference antagonist RWJ-58259, has been designed and synthesized as potential PAR1 antagonists. A DOS strategy has been applied for the synthesis of these urea derivatives, consisting of reduction of basic amino acid-derived amino nitriles, followed by reaction with diverse isocyanates or isothiocyanates. To evaluate the PAR1 antagonist activity, all new synthesized compounds have been screened as inhibitors of human platelet aggregation induced by the PAR1 agonist SFLLRN. Although none of the designed peptide-based ureas inhibited aggregation, some of their protected derivatives showed moderate anti-aggregant activity. These results could be a good clue for the search of new potent PAR1 antagonists and to explore the thrombin/PAR1 interaction.

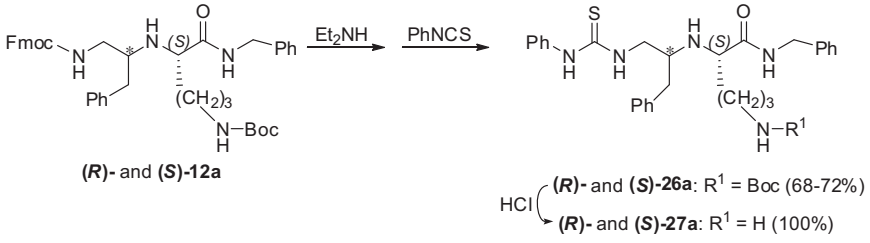
4. Experimental

4.1. General

All reagents were of commercial quality. Solvents were dried and purified by standard methods. Analytical TLC was performed on aluminium sheets coated with a 0.2 mm layer of silica gel 60 F_{254}. Silica gel 60 (230–400 mesh) was used for flash chromatography. Analytical RP-HPLC was performed on a Sunfire C_{18} (4.6 × 150 mm, 3.5 μm) column, with a flow rate of 1 mL/min, using a tunable UV detector set at 214 and 254 nm and a (10–100%, 30 min) gradient of CH_3CN (solvent A) and 0.05% TFA in H_2O (solvent B) as mobile phase. HPLC-MS was performed on a Sunfire C_{18} (4.6 × 50 mm, 3.5 μm) column at 30 °C, with a flow rate of 1 mL/min (10–100%, 5 min) Gradient of CH_3CN with 0.08% of formic acid (solvent A) in 0.1% of formic acid in H_2O (solvent B) was used as mobile phase. Electrospray in positive mode was used for ionization. Melting points were taken on a Mettler Toledo M170 apparatus and are uncorrected. Elemental analyses were obtained on a CH-O-RAOID apparatus. Optical rotations were determined in a Perkin Elmer 141 polarimeter. NMR spectra were recorded using Varian Inova 300, Varian Inova or Mercury 400, and Varian Unity 500 spectrometers. The NMR spectra assignment was based on COSY, HSQC, and HMBC spectra.

4.2. General procedure for the synthesis of basic amino acid-derived ureas. Method A. Synthesis of ureas (*RS*)-*8a,b* and -*9a,b*, and (*R*)- and (*S*)-*9a*

Raney Ni (30 mg) and hydrazine monohydrate (0.186 mL, 6 mmol) were added to a solution of the corresponding amino



Scheme 4. Synthesis of thiourea derivatives.

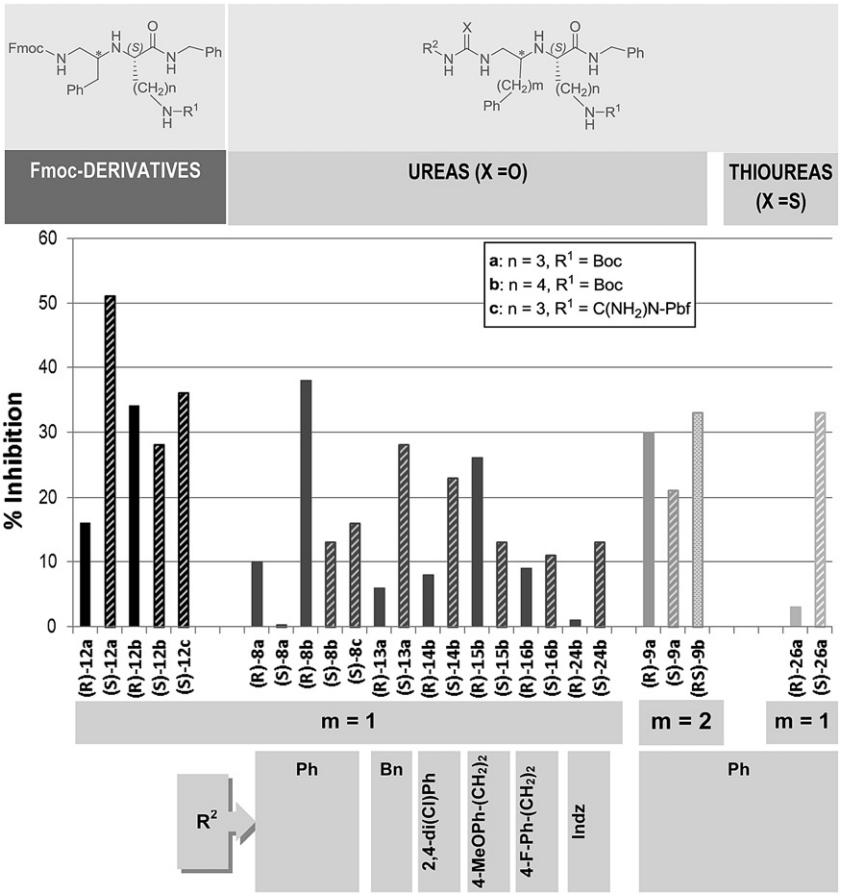


Fig. 2. Inhibition (%) of human platelet aggregation induced by a 30 μM concentration of SFLLRN.

nitrile (***RS***)-**1a**,**b** and (***RS***)-**2a**,**b** (0.3 mmol) in MeOH (20 mL) and the resulting reaction mixture was refluxed for 5 min. Then, the mixture was filtered through celite, this was washed with MeOH (3 × 75 mL), and the filtrates were evaporated to dryness. The residue was dissolved in dry CH_2Cl_2 (10 mL) and the solution was cooled at 0 °C. The corresponding isocyanate (0.3 mmol) was added to the solution that was stirred at this temperature for 2 h. Then, the solvent was evaporated and the residue was dissolved in EtOAc (50 mL). This solution was successively washed with H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using 20–100% EtOAc gradient in hexane as eluant to afford the ureas (***RS***)-**8a**,**b** and (***RS***)-**9a**,**b**. Only both epimers of the ornithine-derived ureas (***R***)-**9a** and (***S***)-**9a** could be separated in this purification. The two epimers of (***RS***)-**8a**,**b** were obtained as optical pure compounds from each epimer of the starting amino nitrile (***R***)-**1a**,**b** and (***S***)-**1a**,**b,** by applying method B described below. The characterization data of (***R***)-**8a**,**b** and (***S***)-**8a**,**b** are also described below.

*4.2.1. N_δ-Boc-N_α-[(2R)-4-phenyl-1-(3-phenylureido)butan-2-yl]-ornithine benzyl amide [(**R**)-**9a**]*

Foam (23 mg, 13%); HPLC t_R 18.54; $[\alpha]_D^{20}$ +7.5 (*c* 0.9, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (s, 9H, Boc), 1.29–1.81 [m, 6H, 3-H (Bu), γ- and β-H (Orn)], 2.49–2.78 [m, 3H, 2-H and 4-H (Bu)], 2.95–3.43 [m, 4H, 1-H (Bu), α- and δ-H (Orn)], 4.32 [m, 2H, CH_2 (Bn)], 4.92 (bs, 1H, *NH*-Boc), 5.86, and 7.67 (2 bs, 2H, HNCONH), 6.95–7.56 (m, 16 H, Ph and *NH*-Bn); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.6 (CH_3, Boc and CH_2, C_γ), 31.6 (CH_2, C_β), 32.3 (CH_2, C_4), 35.7 (CH_2, C_3), 39.5 (CH_2, C_δ), 43.1 (CH_2, C_1), 43.5 [CH_2, (Bn)], 57.1 (CH, C_2), 58.3 (CH, C_α), 79.8 (C, Boc), 119.8–129.2 (15CH, Ph), 138.5, 139.6 and 141.9 (3C, Ph), 156.2 (HNCONH), 157.1 (CO, Boc), 175.1 (CONH); ES-MS *m*/*z* [M + 1]$^+$ calcd. for $C_{34}H_{45}N_5O_4$, 688.35; found 688.17 (100%); Anal. calcd. for $C_{34}H_{45}N_5O_4$: C, 69.48; H, 7.72; N, 11.92. Found: C, 69.67; H, 7.86; N, 11.78.

*4.2.2. N_δ-Boc-N_α-[(2S)-4-phenyl-1-(3-phenylureido)butan-2-yl]-ornithine benzyl amide [(**S**)-**9a**]*

Foam (25 mg, 14%); HPLC t_R 18.89; $[\alpha]_D^{20}$ +5 (*c* 1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9H, Boc), 1.46–1.80 [m, 6H, 3-H (Bu), γ- and β-H (Orn)], 2.51–2.63 [m, 3H, 2-H and 4-H (Bu)], 2.92–3.31 [m, 4H, 1-H (Bu), α- and δ-H (Orn)], 4. 26 [dd, 1H, J = 6 and 15 Hz, CH_2 (Bn)], 4. 31 [dd, 1H, J = 6 and 15 Hz, CH_2 (Bn)], 4.73 (bs, 1H, *NH*-Boc), 5.84, and 7.70 (2 bs, 2H, HNCONH), 6.92–7.52 (m, 16 H, Ph and *NH*-Bn); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.6 (CH_2, C_γ), 28.7 (CH_3, Boc), 32.2 (CH_2, C_β), 32.4 (CH_2, C_4), 35.6 (CH_2, C_3), 40.3 (CH_2, C_δ), 42.5 (CH_2, C_1), 43.4 [CH_2, (Bn)], 57.4 (CH, C_2), 59.9 (CH, C_α), 80.1 (C, Boc), 119.6–129.2 (15CH, Ph), 138.6, 139.6 and 141.9 (3C, Ph), 156.7 (HNCONH), 157.2 (CO, Boc), 174.9 (CONH); ES-MS *m*/*z* [M + 1]$^+$ calcd. for $C_{34}H_{45}N_5O_4$, 688.35; found 688.27 (100%); Anal. calcd. for $C_{34}H_{45}N_5O_4$: C, 69.48; H, 7.72; N, 11.92. Found: C, 69.59; H, 7.82; N, 11.85.

*4.2.3. N_ε-Boc-N_α-[4-phenyl-1-(3-phenylureido)butan-2-yl]-lysine benzyl amide [(**RS**)-**9b**]*

Foam (52 mg, 29%); HPLC t_R 18.70 [(***R***)-**9b**] and 19.06 min [(***S***)-**9b**]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 and 1.43 (2s, 9H, Boc), 0.94–1.33 [m, 2H, δ-H (Lys)], 1.50–1.85 [m, 6H, γ- and β-H (Lys), 3-H (Bu)], 2.45–2.74 [m, 3H, 2-H and 4-H (Bu)], 2.90–3.17 [m, 2H, ε-H (Lys)], 3.17–3.27 [m, 1.5H, 1-H (Bu) and α-H (Lys)], 3.38 [m, 0.5H, 1-H (Bu)],

4.36 [m, 2H, CH_2 (Bn)], 4.66 and 4.84 (2bs, 1H, *NH*-Boc), 5.80, 7.84 and 8.10 (3 bs, 2H, HNCONH), 6.86–7.54 (m, 16 H, Ph and *NH*-Bn); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.9 (CH_2, C_γ), 26.4 and 28.5 (CH_3, Boc), 29.8 (CH_2, C_δ), 32.1 (CH_2, C_4), 33.0 (CH_2, C_β), 34.8 (CH_2, C_3), 40.0 (CH_2, C_ε), 42.0 and 43.0 (CH_2, C_1), 43.3 [CH_2, (Bn)], 56.9 and 58.8 (CH, C_2), 60.1 (CH, C_α), 80.1 (C, Boc), 119.1–128.9 (15CH, Ph), 139.1 and 141.3 (3C, Ph), 156.2 and 156.7 (2CO, Boc and HNCONH), 174.9 (CONH); ES-MS *m/z* $[M + 1]^+$ calcd. for $C_{35}H_{47}N_5O_4$, 602.36; found 602.27 (100%); Anal. calcd. for $C_{35}H_{47}N_5O_4$: C, 69.86; H, 7.87; N, 11.64. Found: C, 69.97; H, 7.84; N, 11.45.

4.3. General procedure for the synthesis of the N-Fmoc-protected amines (*R*)-12a,b and (*S*)-12a–c

Raney Ni (30 mg) and hydrazine monohydrate (0.186 mL, 6 mmol) were added to a solution of the corresponding amino nitrile (*R*)- and (*S*)-**1a**–**c** (0.3 mmol) in MeOH (20 mL) and the resulting reaction mixture was refluxed for 5 min. Then, the mixture was filtered through celite, this was washed with MeOH (3 × 75 mL), and the filtrates were evaporated to dryness. The respective crude reaction mixture of amines (*R*)-**3a**,**b** and (*S*)-**3a**–**c** impurified with **5a**–**c** was dissolved in CH_3CN (10 mL) and cooled at 0 °C. Propylene oxide (56 μL, 8 mmol) and Fmoc-Cl (78 mg, 0.3 mmol) were added. The solution was stirred for 2 h. Then, the solvent was evaporated and the residue was dissolved in EtOAc (50 mL). This solution was successively washed with H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using 10–40% EtOAc gradient in hexane as eluant to give the corresponding Fmoc-protected amine (*R*)-**12a**,**b** and (*S*)-**12a**–**c**.

4.3.1. N_δ-Boc-N_α-[(2R)-3-phenyl-1-(Fmoc)amino-propan-2-yl]-ornithine benzyl amide [(*R*)-12a]

Foam (112 mg, 55%); HPLC t_R 20.56 min; $[\alpha]_D^{20}$ +7.1 (*c* 1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.10–1.98 [m, 4H, β-H and γ-H (Orn)], 1.36 (s, 9H, Boc), 2.76–3.14 [m, 5H, 2-H, 3-H (Pr) and δ-H (Orn)], 3.24 [m, 1H, α-H (Orn)], 3.26–3.55 [m, 1H, 1-H (Pr)], 4.19 [t, 1H, $J = 7$ Hz, CH (Fmoc)], 4.37 [d, 2H, $J = 7$ Hz, CH_2 (Fmoc)], 4.42 [dd, 2H, $J = 5$ and 15 Hz, CH_2 (Bn)], 4.71 (bs, 1H, *NH*-Boc), 5.75 (1bs, 1H, *NH*-Fmoc), 7.14–7.76 (m, 19H, aromatics and *NH*-Bn); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.0(CH_2, C_γ), 28.3 ($3CH_3$, Boc), 30.7 (CH_2, C_β), 39.1 (CH_2, C_3), 39.5 (CH_2, C_δ), 43.1 (CH_2, Bn), 44.6 (CH_2, C_1), 47.1 (CH, Fmoc), 59.6 (CH, C_2), 60.2 (CH, C_α), 66.5 (CH_2, Fmoc), 79.2 (C, Boc), 119.9–143.9 (18CH and 6C, aromatics), 156.2 (CO, Boc), 156.8 (CO, Fmoc), 174.2 (CONH); ES-MS *m/z* $[M + 1]^+$ calcd for $C_{41}H_{48}N_4O_5$, 677.36; found 677.59 (100%); Anal. calcd. for $C_{41}H_{48}N_4O_5$: C, 72.76; H, 7.15; N, 8.28. Found: C, 72.93; H, 7.34; N, 8.05.

4.3.2. N_δ-Boc-N_α-[(2S)-3-phenyl-1-(Fmoc)amino-propan-2-yl]-ornithine benzyl amide [(*S*)-12a]

Foam (162 mg, 80%); HPLC t_R 21.26 min; $[\alpha]_D^{20}$ +15.4 (*c* 1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, 9H, Boc), 1.46–1.59 [m, 4H, β-H and γ-H (Orn)], 2.41–2.71 [m, 2H, 3-H (Pr)], 2.77 [m, 1H, 2-H (Pr)], 2.97–3.16 [m, 2H, δ-H (Orn)], 3.16–3.43 [m, 3H, α-H and 1-H (Orn)], 3.83]dd, 2H, $J = 7$, 15 Hz, CH_2(Bn)], 4.20 [t, 1H, $J = 6.5$ Hz, CH (Fmoc)], 4.35 [dd, 2H, $J = 5$, 15 Hz, CH_2 (Bn)], 4.42 [d, 2H, $J = 6.5$ Hz, CH_2 (Fmoc)], 4.75 (bs, 1H, *NH*-Boc), 5.32 (1bs, 1H, *NH*-Fmoc), 6.91 (1bs, 1H, *NH*-Bn), 7.04–7.77 (m, 19H, aromatics); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.3(CH_2, C_γ), 28.4 ($3CH_3$, Boc), 30.9 (CH_2, C_β), 39.6 (CH_2, C_3), 40.1 (CH_2, C_δ), 42.7 (CH_2, Bn), 43.1 (CH_2, C_1), 47.2 (CH, Fmoc), 58.7 (CH, C_2), 59.1 (CH, C_α), 66.7 (CH_2, Fmoc), 79.3 (C, Boc), 119.9–143.8 (18CH and 6C, aromatics), 156.3 (CO, Boc), 157.1 (CO, Fmoc), 174.2 (CONH); ES-MS *m/z* $[M + 1]^+$ calcd for $C_{41}H_{48}N_4O_5$, 677.36; found 677.59 (100%); Anal. calcd. for $C_{41}H_{48}N_4O_5$: C, 72.76; H, 7.15; N, 8.28. Found: C, 72.89; H, 7.32; N, 8.12.

4.3.3. N_ε-Boc-N_α-[(2R)-3-phenyl-1-(Fmoc)amino-propan-2-yl]-lysine benzyl amide [(*R*)-12b]

Foam (132 mg, 64%); HPLC t_R 20.68 min; $[\alpha]_D^{20}$ +8.2 (*c* 0.8, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91–164 [m, 6H, β-H, γ-H and δ-H (Lys)], 1.44 (s, 9H, Boc), 2.48–2.83 [m, 3H, 2-H (Pr) and ε-H (Lys)], 2.88–3.02 [m, 2H, 3-H (Pr)], 3.06–3.19 [m, 3H, 1-H (Pr) and α-H (Lys)], 4.17 [t, 1H, $J = 7.5$ Hz, CH (Fmoc)], 4.28–4.47 [m, 4H, CH_2 (Fmoc) and CH_2 (Bn)], 4.60 (bs, 1H, *NH*-Boc), 5.18 (1bs, 1H, *NH*-Fmoc), 7.09 (1bs, 1H, *NH*-Bn), 7.11–7.75 (m, 19H, aromatics); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.4 (CH_2, C_γ), 28.3 ($3CH_3$, Boc), 29.6 (CH_2, C_δ), 33.1 (CH_2, C_β), 38.9 (CH_2, C_3), 39.9 (CH_2, C_ε), 42.9 (CH_2, Bn), 44.6 (CH_2, C_1), 47.1 (CH, Fmoc), 59.2 (CH, C_2), 61.1 (CH, C_α), 66.4 (CH_2, Fmoc), 78.9 (C, Boc), 119.8–143.8 (18CH and 6C, aromatics), 155.9 (CO, Boc), 156.7 (NHCOO, Fmoc), 174.4 (CONH); ES-MS *m/z* $[M + 1]^+$ calcd for $C_{42}H_{50}N_4O_5$, 691.38; found 691.59 (100%); Anal. calcd. for $C_{42}H_{50}N_4O_5$: C, 73.02; H, 7.29; N, 8.11. Found: C, 72.89; H, 7.32; N, 8.02.

4.3.4. N_ε-Boc-N_α-[(2S)-3-phenyl-1-(Fmoc)amino-propan-2-yl]-lysine benzyl amide [(*S*)-12b]

Foam (145 mg, 70%); HPLC t_R 21.21 min; $[\alpha]_D^{20}$ +12.3 (*c* 0.9, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.16–1.86 [m, 6H, β-H, γ-H and δ-H (Lys)], 1.42 (s, 9H, Boc), 2.42–2.68 [m, 2H, 3-H (Pr)], 2.72 [m, 1H, 2-H (Pr)], 2.88–3.44 [m, 4H, 1-H (Pr) and ε-H (Lys)], 3.22 [m, 1H, α-H (Lys)], 3.83 [dd, 1H, $J = 7$ and 15 Hz, CH_2 (Bn)], 4.19 [t, 1H, $J = 7.5$ Hz, CH (Fmoc)], 4.35 [dd, 1H, $J = 7$, 15 Hz, CH_2 (Bn)], 4.41 [d, 2H, $J = 7.5$ Hz, CH_2 (Fmoc)], 4.57 (bs, 1H, *NH*-Boc), 5.19 (1bs, 1H, *NH*-Fmoc), 7.04 (1bs, 1H, *NH*-Bn), 7.07–7.77 (m, 19H, aromatics); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.5(CH_2, C_γ), 28.4 ($3CH_3$, Boc), 29.8 (CH_2, C_δ), 33.5 (CH_2, C_β), 39.8 (CH_2, C_3), 41.5 (CH_2, C_ε), 42.7 (CH_2, Bn), 43.5 (CH_2, C_1), 47.2 (CH, Fmoc), 59.3 (CH, C_2), 60.2 (CH, C_α), 66.7 (CH_2, Fmoc), 79.1 (C, Boc), 119.9–143.7 (18CH and 6C, aromatics), 156.2 (CO, Boc), 157.0 (CO, Fmoc), 174.4 (CONH); ES-MS *m/z* $[M + 1]^+$ calcd for $C_{42}H_{50}N_4O_5$, 691.38; found 691.59 (100%) $[M + 1]^+$; Anal. calcd. for $C_{42}H_{50}N_4O_5$: C, 73.02; H, 7.29; N, 8.11. Found: C, 73.14; H, 7.38; N, 7.96.

4.3.5. N_δ-Pbf-N_α-[(2S)-3-phenyl-1-(Fmoc)amino-propan-2-yl]-arginine benzyl amide [(*S*)-12c]

Foam (222 mg, 85%); HPLC [Sunfire C_{18}, 3.9 × 50 mm, 3.5 μm, (10–100%, 5 min) gradient of solvent A in solvent B] t_R 4.65 min; $[\alpha]_D^{20}$ +6.4 (*c* 1, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.12–1.80 [m, 4H, β-H and γ-H (Arg)], 1.45 [s, 6H, 2 CH_3 (Pbf)], 2.09 [s, 3H, CH_3 (Pbf)], 2.51 [s, 3H, CH_3 (Pbf)], 2.59 [s, 3H, CH_3 (Pbf)], 2.66–2.84 [m, 2H, 1-H (Pr)], 2.73 [m, 1H, 2-H (Pr)], 2.92 [s, 2H, CH_2 (Pbf)], 3.02–3.55 [m, 4H, 1-H (Pr) and δ-H(Arg)], 3.38 [m, 1H, α-H (Arg)], 3.85 [dd, 1H, $i = 7.5$ and 15 Hz, CH_2 (Bn)], 4.17 [t, 1H, $J = 7$ Hz, CH_2 (Fmoc)], 4.29 [dd, 1H, $J = 7.5$ and 15 Hz, CH_2 (Bn)], 4.25–4.53 [m, 2H, CH_2 (Fmoc)], 5.60 (bs, 1H, *NH*-Fmoc), 6.28 (bs, 3H, guanidine NH), 6.97–7.78 (m, 15H, aromatics); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 12.9, 18.3, 19.7, 28.9 ($5CH_3$, Pbf), 25.7 (CH_2, C_γ), 30.1 (CH_2, C_β), 31.3 (CH_2, C_3), 40.3 (CH_2, C_δ), 43.1 (CH_2, C_1), 43.6 (CH_2, Pbf), 47.6 (CH, Fmoc), 59.2 (CH, C_2), 59.5 (CH, C_α), 67.1 (CH_2, Fmoc), 86.77, 117.93, 125.0, 132.6, 133.3 (5C, Pbf), 126.9–139.2 (15CH and 5C, aromatics and Pbf), 156.7 (CO, Fmoc), 157.7–159.2 (C=NH, guanidine), 174.8 (CONH); ES-MS *m/z* $[M + 1]^+$ calcd for $C_{50}H_{58}N_6O_6S$: 871.77; found 871.77 (100%); Anal. calcd. for $C_{50}H_{58}N_6O_6S$: C, 68.94; H, 6.71; N, 9.65. Found: C, 69.07; H, 6.82; N, 9.46.

4.4. General procedure for the synthesis of basic amino acid-derived ureas. Method B. Synthesis of ureas (*R*)-8a,b, (*S*)-8a–c, (*R*)- and (*S*)-13a, and (*R*)- and (*S*)-14–16b

Diethyl amine (0.32 mL, 3 mmol) was added to a solution of the corresponding Fmoc-protected amine **12a**–**c** (0.3 mmol) and

the reaction mixture was stirred at rt for 2 h. Then, the solvent was evaporated to dryness and the residue was dissolved in EtOAc (50 mL). The solution was successively washed with H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was dissolved in dry CH_2Cl_2 (10 mL), cooled at 0 °C. The corresponding isocyanate (0.3 mmol) was added to this solution and the mixture was stirred for 15 min–2 h. Then, the solvent was evaporated and the residue was dissolved in EtOAc (50 mL). This solution was successively washed with H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using 20–100% gradient of EtOAc in hexane as eluant to give the corresponding urea (***R***)-**8a**,**b**, (***S***)-**8a**–**c**, (***R***)- and (***S***)-**13a**, and (***R***)- and (***S***)-**14**–**16b**.

4.4.1. N_δ-Boc-N_α-[(2R)-3-phenyl-1-(3-phenylureido)propan-2-yl]-ornithine benzyl amide [(***R***)-***8a***]

Foam (53 mg, 31%); HPLC t_R 17.45 min; $[\alpha]_D^{20}$ +7.2 (*c* 1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.10–2.41 [m, 4H, β-H and γ-H (Orn)], 1.40 (s, 9H, Boc), 2.58–2.76 [m, 2H, 3-H (Pr)], 2.81 [m, 1H, 2-H (Pr)], 2.84–3.02 [m, 5H, 1-H (Pr), α-H and δ-H (Orn)], 4.29 [dd, 1H, J = 6 and 15 Hz, CH_2 (Bn)], 4.41[dd, 1H, J = 6 and 15 Hz, CH_2 (Bn)], 4.62 (bs, 1H, *NH*-Boc), 6.89–7.42 (m, 16H, aromatics and *NH*-Bn), 5.77 and 7.57 (2bs, 2H, NHCONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.0 (CH_2, C_γ), 28.4 ($3CH_3$, Boc), 30.7 (CH_2, C_β), 39.2 (CH_2, C_δ), 39.4 (CH_2, C_3), 43.2 (CH_2, Bn), 43.9 (CH_2, C_1), 60.2 (CH, C_α), 60.4 (CH, C_2), 79.4 (C, Boc), 119.5–139.2 (15CH and 3 C, aromatics), 156.2 (HNCONH), 156.4 (CO, Boc), 175.0 (CONH); ES-MS m/z $[M + 1]^+$ calcd for $C_{33}H_{43}N_5O_4$, 574.33; found 574.27 (100%); Anal. calcd. for $C_{33}H_{43}N_5O_4$: C, 69.08; H, 7.55; N, 12.21. Found: C, 69.13; H, 7.62; N, 12.08.

4.4.2. N_δ-Boc-N_α-[(2S)-3-phenyl-1-(3-phenylureido)propan-2-yl]-ornithine benzyl amide [(***S***)-***8a***]

Foam (144 mg, 84%); HPLC t_R 18.15 min; $[\alpha]_D^{20}$ +6.7 (*c* 1.1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.10–1.74 [m, 4H, β-H and γ-H (Orn)], 1.42 (s, 9H, Boc), 2.40–2.75 [m, 2H, 3-H (Pr)], 2.81–3.14 [m, 2H, 1-H (Pr)], 2.93 [m, 1H, 2-H (Pr)], 3.14–3.48 [m, 3H, α-H and δ-H (Orn)], 3.70 [dd, 1H, J = 7 and 15 Hz, CH_2 (Bn)], 4.20 (dd, 1H, J = 5 and 15 Hz, CH_2 (Bn)], 4.94 (bs, 1H, *NH*-Boc), 6.86–7.47 (m, 15H, aromatics), 6.04 and 8.62 (2bs, 2H, NHCONH), 7.75 (bs, 1H, *NH*-Bn); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.2 (CH_2, C_γ), 28.7 ($3CH_3$, Boc), 30.8 (CH_2, C_β), 40.2 (CH_2, C_δ), 40.6 (CH_2, C_3), 42.4 (CH_2, Bn), 42.9 (CH_2, C_1), 59.5 (CH, C_2), 59.6 (CH, C_α), 79.9 (C, Boc), 119.6–139.6 (15CH and 3C, aromatics), 156.8 (HNCONH), 156.9 (CO, Boc), 174.7 (CONH); ES-MS m/z $[M + 1]^+$ calcd for $C_{33}H_{43}N_5O_4$, 574.33; found 574,52 (100%), 699.43 (10%) $[M + Na]^+$; Anal. calcd. for $C_{33}H_{43}N_5O_4$: C, 69.08; H, 7.55; N, 12.21. Found: C, 69.17; H, 7.60; N, 12.13.

4.4.3. N_ε-Boc-N_α-[(2R)-3-phenyl-1-(3-phenylureido)propan-2-yl]-lysine benzyl amide [(***R***)-***8b***]

Foam (121 mg, 69%); HPLC t_R 18.29 min; $[\alpha]_D^{20}$ +6.5 (*c* 0.6, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.73–1.65 [m, 6H, β-H, γ-H and δ-H (Lys)], 1.37 (s, 9H, Boc), 2.53–2.87 [m, 3H, 3-H and 2-H (Pr)], 2.89–3.00 [m, 2H, 1-H (Pr)], 3.01–3.42 [m, 3H, α-H and ε-H (Lys)], 4.29 [dd, 1H, J = 6 and 15 Hz, CH_2 (Bn)], 4.39 [dd, 1H, J = 6 and 15 Hz, CH_2 (Bn)], 4.59 (s, 1H, *NH*-Boc), 5.51 (bs, 1H, HNCONH), 6.87–7.34 (m, 16H, aromatics and HNCONH), 7.56 (bs, 1H, *NH*-Bn); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.6 (CH_2, C_γ), 28.4 ($3CH_3$, Boc), 29.6 (CH_2, C_δ), 33.3 (CH_2, C_β), 39.0 (CH_2, C_3), 39.9 (CH_2, C_ε), 43.1 (CH_2, Bn), 43.8 (CH_2, C_1), 59.1 (CH, C_2), 61.0 (CH, C_α), 79.3 (C, Boc), 122.8–138.4 (15CH and 3C, aromatics), 156.0 (HNCONH), 156.2 (CO, Boc), 174.0 (CONH); ES-MS m/z $[M + 1]^+$ calcd for $C_{34}H_{45}N_5O_4$, 588.35; found 588.62 (100%); Anal. calcd. for $C_{34}H_{45}N_5O_4$: C, 69.48; H, 7.72; N, 11.92. Found: C, 69.62; H, 7.89; N, 12.75.

4.4.4. N_ε-Boc-N_α-[(2S)-3-phenyl-1-(3-phenylureido)propan-2-yl]-lysine benzyl amide [(***S***)-***8b***]

Foam (143 mg, 81%); HPLC t_R 18.49 min; $[\alpha]_D^{20}$ +4.0 (*c* 0.9, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00–1.90 [m, 6H, β-H, γ-H and δ-H (Lys)], 1.46 (s, 9H, Boc), 2.51–2.68 [m, 2H, 3-H (Pr)], 2.73 [m, 1H, 2-H (Pr)], 2.90–3.18 [m, 2H, ε-H (Lys)], 3.18–3.50 [m, 3H, 1-H (Pr) and α-H (Lys)], 3.90 [dd, 1H, J = 7 and 15 Hz, CH_2 (Bn)], 4.35 [dd, 1H, J = 4 and 15 Hz, CH_2 (Bn)], 4.88 (bs, 1H, *NH*-Boc), 6.82–7.20 (m, 16H, aromatics and *NH*-Bn), 5.98 and 8.09 (2bs, 2H, HNCONH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.1 (CH_2, C_γ), 26.3 ($3CH_3$, Boc), 27.6 (CH_2, C_δ), 27.9 (CH_2, C_β), 30.9 (CH_2, C_3), 38.1 (CH_2, C_ε), 39.8 (CH_2, C_1), 40.7 (CH_2, Bn), 58.0 (CH, C_2), 58.4 (CH, C_α), 77.4 (C, Boc), 117.0–137.3 (15CH and 3 C, aromatics), 154.7 (CO, Boc), 154.8 (HNCONH), 172.1 (CONH); ES-MS m/z $[M + 1]^+$ calcd for $C_{34}H_{45}N_5O_4$, 588.35; found, 588.55 (100%); Anal. calcd. for $C_{34}H_{45}N_5O_4$: C, 69.48; H, 7.72; N, 11.92. Found: C, 69.59; H, 7.84; N, 12.85.

4.4.5. N_ε-Pbf-N_α-[(2S)-3-phenyl-1-(3-phenylureido)propan-2-yl]-arginine benzyl amide [(***S***)-***8c***]

Foam (68 mg, 29%); HPLC t_R 19.95 min; $[\alpha]_D^{20}$ +2.5 (*c* 1, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.61–1.63 [m, 4H, β- and γ-H (Arg)], 1.38, 2.01, 2.39 and 2.45 [4s, 15H, CH_3 (Pbf)], 2.74 [m, 1H, 2-H (Pr)], 2.85 [s, 2H, CH_2 (Pbf)], 2.92–3.17 [m, 4H, 3-H (Pr) and δ-H (Arg)], 3.20–3.42 [m, 2H, 1-H (Pr)], 3.99 [bs, 1H, α-H (Arg)], 4.17 [d, 1H, J = 6 and 14.5 Hz, CH_2 (Bn)], 4.30 [dd, 1H, J = 4.5 and 14.5 Hz, CH_2 (Bn)], 6.20 [bs, 3H, guanidino], 6.85 (t, 1H, J = 6 Hz, *NH*-Bn), 7.00–7.34 (m, 15H, aromatics), 7.78 and 8.15 (2bs, 2H, NHCONH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.9, 18.3, 19.7, 28.9 (CH_3, Pbf), 25.7 (CH_2, C_γ), 30.1 (CH_2, C_β), 31.3 (CH_2, C_3), 40.3 (CH_2, C_δ), 43.6 (CH_2, C_1), 43.6 (CH_2, Pbf), 59.2 (CH, C_2), 59.5 (CH, C_α), 86.77, 117.93, 125.0, 132.6, 133.3 (5C, Pbf), 126.9–139.2 [15CH and 4C, aromatics and Pbf], 156.4 (HNCONH), 159.2 (guanidino); ES-MS m/z $[M + 1]^+$ calcd. for $C_{42}H_{53}N_7O_5S$, 767.38; found, 768.81 (100%). Anal. calcd. for $C_{42}H_{53}N_7O_5S$: C, 65.69; H, 6.96; N, 12.77. Found: C, 65.82; H, 7.04; N, 12.68.

4.4.6. N_δ-Boc-N_α-[(2R)-1-(3-benzylureido)-3-phenylpropan-2-yl]-ornithine benzyl amide [(***R***)-***13a***]

Foam (80 mg, 45%); HPLC t_R 18.47 min; $[\alpha]_D^{20}$ +6.5 (*c* 1.1, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.19–1.69 [m, 4H, β-H and γ-H (Orn)], 1.39 (s, 9H, Boc), 2.61–2.65 [m, 2H, 3-H (Pr)], 2.80 [m, 1H, 2-H (Pr)], 2.87–3.20 [m, 4H, 1-H (Pr), and δ-H (Orn)], 3.21 [m, 1H, α-H (Orn)], 4.27 [m, 2H, CH_2 (Bn-ureido)], 4.32 [dd, 1H, J = 6 and 15 Hz, CH_2 (Bn)], 4.40 [dd, 1H, J = 6 and 15 Hz, CH_2 (Bn)], 4.58 (bs, 1H, *NH*-Boc), 4.73 and 5.06 (2bs, 2H, NHCONH), 7.07–7.36 (m, 16H, aromatics and *NH*-Bn); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.1 (CH_2, C_γ), 28.4 ($3CH_3$, Boc), 30.6 (CH_2, C_β), 39.4 (CH_2, C_3), 39.5 (CH_2, C_δ), 43.2 (CH_2, Bn), 43.9 [CH_2, (Bn-ureido)], 44.4 (CH_2, C_1), 59.4 (CH, C_2), 59.9 (CH, C_α), 79.4 (C, Boc), 127.2–138.7 (15CH and 3 C, aromatics), 156.2 (CO, Boc), 158.2 (HNCONH), 174.6 (CONH); ES-MS m/z $[M + 1]^+$ calcd for $C_{34}H_{45}N_5O_4$, 588.35; found 588.48 (100%); Anal. calcd. for $C_{34}H_{45}N_5O_4$: C, 69.48; H, 7.72; N, 11.92. Found: C, 69.61; H, 7.62; N,12.08.

4.4.7. N_δ-Boc-N_α-[(2S)-1-(3-benzylureido)-3-phenylpropan-2-yl]-ornithine benzyl amide [(***S***)-***13a***]

Foam (112 mg, 64%); HPLC t_R 18.86 min; $[\alpha]_D^{20}$ +7.0 (*c* 0.9, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (s, 9H, Boc), 1.41–1.85 [m, 4H, β-H and γ-H (Orn)], 2.45–2.81 [m, 2H, 3-H (Pr)], 2.74 [m, 1H, 2-H (Pr)], 2.86–3.34 [m, 4H, 1-H (Pr) and δ-H (Orn)], 3.42 [m, 2H, α-H (Orn)], 3.78 [dd, 1H, J = 7 and 15 Hz, CH_2 (Bn)], 4.30 (dd, 1H, J = 4 and 15 Hz, CH_2 (Bn)], 4.37 [m, 2H, CH_2 (Bn-ureido)], 4.94 (bs, 1H, *NH*-Boc), 6.86–7.47 (m, 15H, aromatics), 5.81 (1bs, 2H, NHCONH), 6.96 (bs, 1H, *NH*-Bn); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.8 (CH_2, C_γ), 28.3 ($3CH_3$, Boc), 30.5 (CH_2, C_β), 39.8 (CH_2, C_δ), 40.3 (CH_2, C_3), 41.7 (CH_2, Bn), 42.6 [CH_2, (Bn-ureido)], 44.3 (CH_2, C_1), 58.6 (CH, C_2), 59.2 (CH, C_α), 79.7 (C, Boc), 126.3–139.7 (15CH and 3C, aromatics), 156.7

(CO, Boc), 159.2 (HNCONH), 174.4 (CONH); ES-MS *m/z* [M + 1]$^+$ calcd for $C_{34}H_{45}N_5O_4$, 588.35; found 588.62 (100%); Anal. calcd. for $C_{34}H_{45}N_5O_4$: C, 69.48; H, 7.72; N, 11.92. Found: C, 69.63; H, 7.84; N, 11.87.

*4.4.8. N_ε-Boc-N_α-[(2R)-1-(3-(2,4-dichlorophenyl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide [(**R**)-**14b**]*

Foam (125 mg, 63%); HPLC t_R 20.05 min; $[\alpha]_D^{20}$ +7.5 (*c* 1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.73–1.65 [m, 6H, β-H, γ-H and δ-H (Lys)], 1.42 (s, 9H, Boc), 2.63 [dd, 1H, *J* = 6 and 13.5 Hz, 3-H (Pr)], 2.72 [dd, 1H, *J* = 6 and 13.5 Hz, 3-H (Pr)], 2.83–3.14 [m, 5H, 1-H (Pr), 2-H (Pr), and ε-H (Lys)], 3.32 [m, 1H, α-H (Lys)], 4.30 [dd, 1H, *J* = 6 and 15 Hz, CH_2 (Bn)], 4.40 [dd, 1H, *J* = 6 and 15 Hz, CH_2 (Bn)], 4.55 (s, 1H, *NH*-Boc), 5.96 (bs, 2H, HNCONH), 7.04–7.31 (m, 13H, aromatics), 7.33 (bs, 1H, *NH*-Bn); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.5 (CH_2, C_γ), 28.4 ($3CH_3$, Boc), 29.6 (CH_2, C_δ), 33.6 (CH_2, C_β), 39.2 (CH_2, C_3), 39.8 (CH_2, C_ε), 43.2 (CH_2, Bn), 43.9 (CH_2, C_1), 58.9 (CH, C_2), 60.8 (CH, C_α), 79.4 (C, Boc), 121.5–138.2 (13CH and 5C, aromatics), 154.9 (HNCONH), 156.3 (CO, Boc), 175.0 (CONH); ES-MS *m/z* [M + 1]$^+$ calcd for $C_{34}H_{43}Cl_2N_5O_4$, 656.27; found, 656.66 (100%), 658.62 (70%) [M + 3]$^+$, 660.65 (10%) [M + 5]$^+$; Anal. calcd. for $C_{34}H_{43}Cl_2N_5O_4$: C, 62.19; H, 6.60; N, 10.67. Found: C, 62.32; H, 6.75; N, 10.48.

*4.4.9. N_ε-Boc-N_α-[(2S)-1-(3-(2,4-dichlorophenyl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide [(**S**)-**14b**]*

Foam (147 mg, 74%); HPLC t_R 20.03 min; $[\alpha]_D^{20}$ +3.0 (*c* 0.8, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86–2.19 [m, 6H, β-H, γ-H and δ-H (Lys)], 1.45 (s, 9H, Boc), 2.66–2.91 [m, 3H, 2-H and 3-H (Pr)], 3.00–3.54 [m, 5H, 1-H (Pr), α-H and ε-H (Lys)], 4.00 [dd, 1H, *J* = 6.5 and 15 Hz, CH_2 (Bn)], 4.37 [dd, 1H, *J* = 6.5 and 15 Hz, CH_2 (Bn)], 4.86 (s, 1H, *NH*-Boc), 6.33 (bs, 2H, HNCONH), 6.90–7.58 (m, 13H, aromatics), 7.70 (bs, 1H, *NH*-Bn); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.8 (CH_2, C_γ), 28.5 ($3CH_3$, Boc), 29.7 (CH_2, C_β and C_δ), 36.6 (CH_2, C_3), 39.7 (CH_2, C_ε), 40.0 (CH_2, Bn), 42.9 (CH_2, C_1), 60.2 (CH, C_2), 60.4 (CH, C_α), 79.9 (C, Boc), 121.4–138.5 (13CH and 5C, aromatics), 154.7 (CO, Boc),157.2 (HNCONH), 170.2 (CONH); ES-MS *m/z* [M + 1]$^+$ calcd for $C_{34}H_{43}Cl_2N_5O_4$, 656.27; found, 656.46 (100%), 658.48 (80%) [M + 3]$^+$, 660.49 (15%) [M + 5]$^+$; Anal. calcd. for $C_{34}H_{43}Cl_2N_5O_4$: C, 62.19; H, 6.60; N, 10.67. Found: C, 62.29; H, 6.71; N, 10.55.

*4.4.10. N_ε-Boc-N_α-[(2R)-1-(3-(4-methoxyphenethyl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide [(**R**)-**15b**]*

Foam (127 mg, 66%); HPLC t_R 18.29 min; $[\alpha]_D^{20}$ +8.5 (*c* 1.1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78–1.71 [m, 6H, β-H, γ-H and δ-H (Lys)], 1.42 (s, 9H, Boc), 2.66 [t, 2H, *J* = 7 Hz, 2-H (ethyl)], 2.66–2.82 [m, 3H, 2-H and 3-H (Pr)], 2.91–3.18 [m, 5H, 1-H (Pr), α-H and ε-H (Lys)], 3.28 [dd, 2H, *J* = 7 and 13 Hz, 1-H (ethyl)], 3.76 (s, 3H, OMe), 4.32 [dd, 1H, *J* = 6 and 14.5 Hz, CH_2 (Bn)], 4.41 [dd, 1H, *J* = 6 and 14.5 Hz, CH_2 (Bn)], 4.55 (bs, 1H, *NH*-Boc), 4.70 (bs, 2H, HNCONH), 7.04–7.33 (m, 14H, aromatics), 7.57 (bs, 1H, *NH*-Bn); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.6 (CH_2, C_γ), 28.4 ($3CH_3$, Boc), 29.7 (CH_2, C_δ), 30.0 (CH_2, C_β), 35.4 (CH_2, C_3), 35.5 [CH_2, C_2 (ethyl)], 38.9 [CH_2, C_ε and C_1 (ethyl)], 43.1 (CH_2, Bn), 43.9 (CH_2, C_1), 55.2 (CH_3, OMe), 59.5 (CH, C_2), 60.9 (CH, C_α), 79.1 (C, Boc), 113.9–138.5 (14CH and 4C, aromatics), 156.0 (CO, Boc), 158.3 (HNCONH), 174.6 (CONH); ES-MS *m/z* [M + 1]$^+$ calcd for $C_{37}H_{51}N_5O_5$, 646.39; found, 646.72 (100%); Anal. calcd. for $C_{37}H_{51}N_5O_5$: C, 68.81; H, 7.96; N, 10.84. Found: C, 68.96; H, 8.07; N, 10.48.

*4.4.11. N_ε-Boc-N_α-[(2S)-1-(3-(4-methoxyphenethyl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide [(**S**)-**15b**]*

Foam (143 mg, 87%); HPLC t_R 18.43 min; $[\alpha]_D^{20}$ +5.9 (*c* 1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.09–2.23 [m, 6H, β-H, γ-H and δ-H (Lys)], 1.43 (s, 9H, Boc), 2.60–2.69 [m, 2H, 3-H (Pr)], 2.65–2.72 [m, 2H, 2-H (Pr)], 2.76 [t, 2H, *J* = 7 Hz, 2-H (ethyl)], 2.99–3.17 [m, 4H, 1-H (Pr) and ε-H (Lys)], 3.29–3.50 [m, 1H, α-H (Lys)], 3.40 [dd, 2H, *J* = 7 and 13 Hz, 1-H (ethyl)], 3.80 (s, 3H, OMe), 3.91 [dd, 1H, *J* = 7 and 15 Hz, CH_2 (Bn)], 4.35 [dd, 1H, *J* = 7 and 15 Hz, CH_2 (Bn)], 4.85 (bs, 1H, *NH*-Boc), 5.15 and 5.29 (2bs, 2H, HNCONH), 6.75–7.44 (m, 15H, aromatics and *NH*-Bn); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.6 (CH_2, C_γ), 28.8 ($3CH_3$, Boc), 30.1 (CH_2, C_δ), 30.5 (CH_2, C_β), 33.5 (CH_2, C_3), 35.5 [CH_2, C_2 (ethyl)], 40.5 (CH_2, C_ε), 40.8 [CH_2, C_1 (ethyl)], 42.2 (CH_2, Bn), 43.1 (CH_2, C_1), 55.6 (CH_3, OMe), 60.6 (CH, C_2), 60.8 (CH, C_α), 79.9 (C, Boc), 114.3–139.5 (14CH and 4C, aromatics), 157.1 (CO, Boc), 159.5 (HNCONH), 174.9 (CONH); ES-MS *m/z* [M + 1]$^+$ calcd for $C_{37}H_{51}N_5O_5$, 646.39; found, 646.52 (100%); Anal. calcd. for $C_{37}H_{51}N_5O_5$: C, 68.81; H, 7.96; N, 10.84. Found: C, 68.93; H, 8.01; N, 10.69.

*4.4.12. N_ε-Boc-N_α-[(2R)-1-((3-(4-fluorophenethyl)ureido)-3-phenyl)propan-2-yl]-lysine benzyl amide [(**R**)-**16b**]*

Foam (118 mg, 63%); HPLC t_R 18.47 min; $[\alpha]_D^{20}$ +7.6 (*c* 1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.81–2.11 [m, 6H, β-H, γ-H and δ-H (Lys)], 1.46 (s, 9H, Boc), 2.56–2.89 [m, 3H, 2-H and 3-H (Pr)], 2.77 [t, 2H, *J* = 7 Hz, 2-H (ethyl)], 2.93–3.28 [m, 5H, 1-H (Pr), α-H and ε-H (Lys)], 3.32 [dd, 2H, *J* = 7 and 13 Hz, 1-H (ethyl)], 4.41 [d, 2H, *J* = 7 Hz, CH_2 (Bn)], 4.58 (bs, 1H, *NH*-Boc), 4.80 (bs, 2H, HNCONH), 6.89–7.48 (m, 14H, aromatics), 7.52 (bs, 1H, *NH*-Bn); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.3 (CH_2, C_γ), 28.0 ($3CH_3$, Boc), 29.3 (CH_2, C_δ), 30.5 (CH_2, C_β), 32.9 [CH_2, C_2 (ethyl)], 35.2 (CH_2, C_3), 38.9 (CH_2, C_ε), 41.2 [CH_2, C_1 (ethyl)], 42.7 (CH_2, Bn), 43.8 (CH_2, C_1), 59.1 (CH, C_2), 60.7 (CH, C_α), 79.8 (C, Boc), 114.7–159.5 (14CH and 4C, aromatics), 155.7 (CO, Boc), 157.9 (HNCONH), 174.4 (CONH); ES-MS *m/z* [M + 1]$^+$ calcd for $C_{36}H_{48}FN_5O_4$, 634.37; found, 634.68 (100%); Anal. calcd. for $C_{36}H_{48}FN_5O_4$: C, 68.22; H, 7.63; N, 11.05. Found: C, 68.38; H, 7.75; N, 10.89.

*4.4.13. N_ε-Boc-N_α-[(2S)-1-((3-(4-fluorophenethyl)ureido)-3-phenyl)propan-2-yl]-lysine benzyl amide [(**S**)-**16b**]*

Foam (168 mg, 89%); HPLC t_R 18.57 min; $[\alpha]_D^{20}$ +3.5 (*c* 1.1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.77–2.23 [m, 6H, β-H, γ-H and δ-H (Lys)], 1.45 (s, 9H, Boc), 2.58–2.73 [m, 3H, 2-H and 3-H (Pr)], 2.73 [t, 2H, *J* = 7 Hz, 2-H (ethyl)], 2.94–3.28 [m, 4H, 1-H (Pr) and ε-H (Lys)], 3.28–3.37 [m, 1H, α-H (Lys)], 3.40 [dd, 2H, *J* = 7 and 13 Hz, 1-H (ethyl)], 3.89 [dd, *J* = 7 and 15 Hz, CH_2 (Bn)], 4.34 [dd, *J* = 7 and 15 Hz, CH_2 (Bn)], 4.88 (bs, 1H, *NH*-Boc), 5.26 and 5.41 (2bs, 2H, HNCONH), 6.88–7.60 (m, 15H, aromatics and *NH*-Bn); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.6 (CH_2, C_γ), 28.8 ($3CH_3$, Boc), 30.1 (CH_2, C_δ), 30.5 (CH_2, C_β), 33.6 [CH_2, C_2 (ethyl)], 36.2 (CH_2, C_3), 40.5 (CH_2, C_ε), 40.9 [CH_2, C_1 (ethyl)], 42.0 (CH_2, Bn), 43.1 (CH_2, C_1), 60.6 (CH, C_2), 60.8 (CH, C_α), 79.8 (C, Boc), 115.5–139.6 (14CH and 4C, aromatics), 157.1 (CO, Boc), 160.3 (HNCONH), 175.0 (CONH); ES-MS *m/z* [M + 1]$^+$ calcd for $C_{36}H_{48}FN_5O_4$, 634.37; found, 634.43 (100%); Anal. calcd. for $C_{36}H_{48}FN_5O_4$: C, 68.22; H, 7.63; N, 11.05. Found: C, 68.36; H, 7.78; N, 10.95.

*4.5. General procedure for the N-Boc-deprotection of protected ureas (**RS**)-**9b**, (**R**)- and (**S**)-**8a,b**, (**R**)-, (**S**)-**9a** and -**13a**, (**R**)- and (**S**)-**14**–**16b**. Synthesis of urea hydrochlorides (**R**)- and (**S**)-**11a**, (**RS**)-**11b**, (**R**)- and (**S**)-**17a,b**, (**R**)- and (**S**)-**18a**, (**R**)- and (**S**)-**19**–**21b***

The corresponding protected urea (0.10 mmol) was dissolved in a 3 M solution of HCl in EtOAc (2 mL) and stirred at rt for 2 h. Then, the solvent was evaporated under reduced pressure, the residue was dissolved in H_2O (3 mL) and the solution was lyophilized.

*4.5.1. N_α-[(2R)-4-Phenyl-1-(3-phenylureido)butan-2-yl]-ornithine benzyl amide dihydrochloride [(**R**)-**11a**]*

Amorphous solid (56 mg, 100%); $[\alpha]_D^{20}$ +6.9 (*c* 0.9, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.51–2.06 [m, 6H, 3-H (Bu), γ- and β-H (Orn)], 2.64–2.94 [m, 4H, 4-H (Bu) and δ-H (Orn)], 3.08 [m, 1H, 2-H

(Bu)], 3.22–3.45 [m, 2H, 1-H (Bu)], 4.09 [m, 1H, α-H (Orn)], 4.25 [dd, 1H, J = 5 and 15 Hz, CH_2 (Bn)], 4.40 [dd, 1H, J = 4 and 15 Hz, CH_2 (Bn)], 6.74 and 6.91 (2bs, 2H, HNCONH), 6.97–7.47 (m, 15H, Ph), 7.95 [bs, 3H, δ – NH_3^+ (Orn)], 9.11 and 9.43 [2bs, 2H, α – NH_2^+ (Orn)], 9.33 (bs, 1H, *NH*-Bn); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.7 (CH_2, C_γ), 27.2 (CH_2, C_β), 28.1 (CH_2, C_4), 29.4 (CH_2, C_3), 31.0 (CH_2, C_δ), 38.1 [CH_2, (Bn)], 42.6 (CH_2, C_1), 56.6 (CH, C_2), 57.8 (CH, C_α), 118.7–140.7 (15CH, and 3C, Ph), 156.5 (HNCONH), 166.9 (CONH); ES-MS m/z [M + 1]$^+$ calcd. for $C_{29}H_{37}N_5O_2$, 488.29; found, 488.50 (100%).

4.5.2. N_α-[(2S)-4-Phenyl-1-(3-phenylureido)butan-2-yl]-ornithine benzyl amide dihydrochloride [(***S***)-***11a***]

Amorphous solid (55 mg, 100%); $[\alpha]_D^{20}$ +11.3 (*c* 1, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.61–2.09 [m, 6H, 3-H (Bu), γ- and β-H (Orn)], 2.55–2.96 [m, 4H, 4-H (Bu) and δ-H (Orn)], 3.05 [m, 1H, 2-H (Bu)], 3.35–3.71 [m, 2H, 1-H (Bu)], 4.25 [m, 1H, α-H (Orn)], 4.33 [d, 2H, J = 5, CH_2 (Bn)], 6.92 and 7.32 (2bs, 2H, HNCONH), 7.12–7.64 (m, 15H, Ph), 8.07 [bs, 3H, δ – NH_3^+ (Orn)], 9.11 and 9.43 [2bs, 2H, α – NH_2^+ (Orn)], 9.19 (bs, 1H, *NH*-Bn); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.4 (CH_2, C_γ), 27.1 (2CH_2, C_4 and C_β), 30.5 (CH_2, C_3), 30.9 (CH_2, C_δ), 37.8 [CH_2, (Bn)], 42.4 (CH_2, C_1), 57.2 (CH, C_2), 57.5 (CH, C_α), 117.7–139.9 (15CH, and 3C, Ph), 156.0 (HNCONH), 166.5 (CONH); ES-MS m/z [M + 1]$^+$ calcd. for $C_{29}H_{37}N_5O_2$, 488.29; found, 488.50 (100%).

4.5.3. N_α-[4-Phenyl-1-(3-phenylureido)butan-2-yl]-lysine benzyl amide dihydrochloride [(***RS***)-***11b***]

Amorphous solid (57 mg, 29%); HPLC t_R 13.26 [(***R***)-**9b**] and 13.45 min [(***S***)-**9b**]; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.23–1.40 [m, 2H, γ-H (Lys)], 1.43–1.62 [m, 2H, β-H (Lys)], 1.70–1,96 [m, 4H, δ-H (Lys) and 3-H (Bu)], 2.58–2.78 [m, 4H, 4-H (Bu) and ε-H (Lys)], 2.91–3.06 [m, 1H, 2-H (Bu)], and α-H (Lys)], 3.38–3.65 [m, 2H, 1-H (Bu)], 4.03 and 4.16 [2m, 1H, α-H (Lys)], 4.26 [dd, 0.5H, J = 7 and 15 Hz, CH_2 (Bn)], 4.30 [d, 1H, J = 5 Hz, CH_2 (Bn)], 4.35 [dd, 0.5H, J = 7 and 15 Hz, CH_2 (Bn)], 6.77 (bs, 2H, HNCONH), 7.10–7.43 (m, 15 H, Ph), 7.92 [bs, 3H, δ – NH_3^+ (Lys)], 8.93, 8.77, 9.33 and 9.46 [4bs, 2H, α – NH_2^+ (Lys)], 9.32 (bs, 1H, *NH*-Bn); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.9 (CH_2, C_γ), 29.8 (CH_2, C_δ), 32.1 (CH_2, C_4), 33.0 (CH_2, C_β), 34.8 (CH_2, C_3), 40.0 (CH_2, C_ε), 42.0 and 43.0 (CH_2, C_1), 43.3 [CH_2, (Bn)], 56.9 and 58.8 (CH, C_2), 60.1 (CH, C_α), 119.1–141.3 (15CH, and 3C, Ph), 156.2 and 156.7 (HNCONH), 174.9 (CONH); ES-MS m/z [M + 1]$^+$ calcd. for $C_{30}H_{39}N_5O_2$, 502.31; found, 502.50 (100%).

4.5.4. N_α-[(2R)-3-Phenyl-1-(3-phenylureido)propan-2-yl]-ornithine benzyl amide dihydrochloride [(***R***)-***17a***]

Amorphous solid (54 mg, 100%); HPLC t_R 12.87 min; $[\alpha]_D^{20}$ +5.5 (*c* 1.3, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.56–1.79 [m, 2H, γ-H (Orn)], 1.80–2.07 [m, 2H, β-H (Orn)], 2.69–3.31 [m, 7H, 1-H, 2-H, 3-H (Pr) and δ-H(Orn)], 4.25 [dd, 1H, J = 6 and 15 Hz, CH_2 (Bn)], 4.33 [m, 2H, α-H (Orn)], 4.50 [dd, 1H, J = 6 and 15 Hz, CH_2 (Bn)], 6.89 (bs, 2H, NHCONH), 6.79–7.48 (m, 15H, aromatics), 8.08 [bs, 3H, δ – NH_3^+ (Orn)], 9.15 and 9.78 [2bs, 2H, α – NH_2^+ (Orn)], 9.65 (bs, 1H, *NH*-Bn); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.1 (CH_2, C_γ), 27.1 (CH_2, C_β), 28.2 (CH_2, C_3), 33.5 (CH_2, C_δ), 38.0 (CH_2, Bn), 42.6 (CH_2, C_1), 56.7 (CH, C_2), 59.5 (CH, C_α), 118.1–148.0 (15CH and 3C, aromatics), 156.2 (HNCONH), 167.0 (CONH); ES-MS m/z [M + 1]$^+$ calcd for $C_{28}H_{35}N_5O_2$, 474.28; found, 574.27 (100%).

4.5.5. N_α-[(2S)-3-Phenyl-1-(3-phenylureido)propan-2-yl]-ornithine benzyl amide dihydrochloride [(***S***)-***17a***]

Amorphous solid (55 mg, 100%); HPLC t_R 12.89 min; $[\alpha]_D^{20}$ + 26.6 (*c* 0.9, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.67–1.78 [m, 2H, γ-H (Orn)], 1.82–2.06 [m, 2H, β-H (Orn)], 2.74–2.96 [m, 3H, 3-H (Pr) and δ-H (Orn)], 2.99–3.18 [m, 1H, 3-H (Pr)], 3.11 [m, 1H, 1-H (Pr)], 3.23 [m, 1H, 2-H (Pr)], 3.53 [m, 1H, 1-H (Pr)], 4.27–4.44 [m, 1H, α-H (Orn)], 4.35 [d, 2H, J = 6.5 Hz, CH_2 (Bn)], 6.86–7.45 (m, 15H, aromatics), 6.94 and 7.10 (2bs, 2H, NHCONH), 8.12 [bs, 3H, δ – NH_3^+ (Orn)], 9.41 and 9.50 [2bs, 2H, α – NH_2^+ (Orn)], 9.64 (bs, 1H, *NH*-Bn); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.7 (CH_2, C_γ), 27.3 (CH_2, C_β), 34.9 (CH_2, C_3), 37.7 (CH_2, C_δ), 38.8 (CH_2, Bn), 42.7 (CH_2, C_1), 57.7 (CH, C_2), 59.0 (CH, C_α), 117.8–140.2 (15CH and 3 C, aromatics), 156.2 (HNCONH), 167.2 (CONH); ES-MS m/z [M + 1]$^+$ calcd for $C_{28}H_{35}N_5O_2$, 474.28; found, 574.52 (100%).

4.5.6. N_α-[(2R)-3-Phenyl-1-(3-phenylureido)propan-2-yl]-lysine benzyl amide dihydrochloride [(***R***)-***17b***]

Amorphous solid (56 mg, 100%); HPLC t_R 12.78 min; $[\alpha]_D^{20}$ +16.5 (*c* 1.6, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25–1.48 [m, 2H, γ-H (Lys)], 1.50–1.70 [m, 2H, δ-H (Lys)], 1.72–2.08 [m, 2H, β-H (Lys)], 2.72 [m, 2H, ε-H (Lys)], 2.77–2.92 [m, 1H, 3-H (Pr)], 3.08–3.34 [m, 3H, 1-H and 3-H (Pr)], 3.24 [m, 1H, 2-H (Pr)], 4.20 [m, 1H, α-H (Lys)], 4.28 [dd, 1H, J = 6 and 15 Hz, CH_2 (Bn)], 4.45 [dd, 1H, J = 5 and 15 Hz, CH_2 (Bn)], 6.89 (bs, 2H, HNCONH), 7.09–7.49 (m, 15H, aromatics), 8.01 [bs, 3H, δ – NH_3^+ (Lys)], 9.01 and 9.77 [2bs, 2H, α – NH_2^+ (Lys)], 9.47 (bs, 1H, *NH*-Bn); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.0 (CH_2, C_γ), 27.1 (CH_2, C_δ), 30.2 (CH_2, C_β), 34.3 (CH_2, C_3), 38.9 (CH_2, C_ε), 38.9 (CH_2, Bn), 43.3 (CH_2, C_1), 57.9 (CH, C_2), 60.3 (CH, C_α), 118.9–140.7 (15CH and 3C, aromatics), 157.3 (HNCONH), 167.8 (CONH); ES-MS m/z [M + 1]$^+$ calcd for $C_{29}H_{37}N_5O_2$, 488.29; found, 488.50 (100%).

4.5.7. N_α-[(2S)-3-Phenyl-1-(3-phenylureido)propan-2-yl]-lysine benzyl amide dihydrochloride [(***S***)-***17b***]

Amorphous solid (57 mg, 100%); HPLC t_R 13.26 min; $[\alpha]_D^{20}$ +19.3 (*c* 1.5, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.28–1.49 [m, 2H, γ-H (Lys)], 1.51–1.69 [m, 2H, δ-H (Lys)], 1.74–2.01 [m, 2H, β-H (Lys)], 2.69 [m, 2H, ε-H (Lys)], 2.78–2.94 [m, 1H, 3-H (Pr)], 2.99–3.18 [m, 2H, 1-H and 3-H (Pr)], 3.23 [m, 1H, 2-H (Pr)], 3.51–3.60 [m, 1H, 1-H (Pr)], 4.23–4.48 [m, 1H, α-H (Lys)], 4.33 [d, 2H, J = 5 Hz, CH_2 (Bn)], 6.80–7.48 [m, 18H, aromatics and δ – NH_3^+ (Lys)], 6.89 and 7.15 (2bs, 2H, NHCONH), 9.09 and 9.51 [2bs, 2H, α – NH_2^+ (Lys)], 9.51 (bs, 1H, *NH*-Bn); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.1 (CH_2, C_γ), 27.0 (CH_2, C_δ), 30.4 (CH_2, C_β), 35.7 (CH_2, C_3), 38.5 (CH_2, C_ε), 38.9 (CH_2, Bn), 43.3 (CH_2, C_1), 58.9 (CH, C_2), 59.8 (CH, C_α), 118.5–140.9 (15CH and 3C, aromatics), 157.0 (HNCONH), 168.0 (CONH); ES-MS m/z [M + 1]$^+$ calcd for $C_{29}H_{37}N_5O_2$, 488.29; found, 488.50 (100%).

4.5.8. N_α-[(2R)-1-(3-Benzylureido)-3-phenylpropan-2-yl]-ornithine benzyl amide dihydrochloride [(***R***)-***18a***]

Amorphous solid (56 mg, 100%); HPLC t_R 12.87 min; $[\alpha]_D^{20}$ +5.2 (*c* 1, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.56–1.82 [m, 2H, γ-H (Orn)], 1.90–2.10 [m, 2H, β-H (Orn)], 2.74–3.02 [m, 4H, 3-H (Pr) and δ-H (Orn)], 3.23 [m, 1H, 2-H (Pr)], 3.09–3.22 [m, 2H, 1-H (Pr)], 4.30 [m, 2H, CH_2 (Bn-ureido)], 4.32–4.38 [m, 1H, α-H (Orn)], 4.35 [dd, 1H, J = 6.5 and 15 Hz, CH_2 (Bn)], 4.60 [dd, 1H, J = 5 and 15 Hz, CH_2 (Bn)], 6.72 and 7.00 (2bs, 2H, NHCONH), 7.21–7.65 (m, 15H, aromatics), 8.08 [bs, 3H, δ – NH_3^+ (Orn)], 9.56 and 9.81 [2bs, 2H, α – NH_2^+ (Orn)], 9.56 (bs, 1H, *NH*-Bn); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.7 (CH_2, C_γ), 27.2 (CH_2, C_β), 28.9 (CH_2, C_3), 33.5 (CH_2, C_δ), 38.1 (CH_2, Bn), 42.6 [CH_2, (Bn-ureido)], 43.1 (CH_2, C_1), 56.7 (CH, C_2), 60.4 (CH, C_α), 126.9–140.2 (15CH and 3C, aromatics), 159.7 (HNCONH), 166.9 (CONH); ES-MS m/z [M + 1]$^+$ calcd for $C_{29}H_{37}N_5O_2$, 488.29; found, 488.52 (100%).

4.5.9. N_α-[(2S)-1-(3-Benzylureido)-3-phenylpropan-2-yl]-ornithine benzyl amide dihydrochloride [(***S***)-***18a***]

Amorphous solid (55 mg, 100%); HPLC t_R 12.95 min; $[\alpha]_D^{20}$ +2.1 (*c* 1.5, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.58–1.74 [m, 2H, γ-H (Orn)], 1.80–1.99 [m, 2H, β-H (Orn)], 2.74–3.10 [m, 5H, 1-H, 3-H (Pr) and δ-H (Orn)], 3.18 [m, 1H, 2-H (Pr)], 3.38–3.46 [m, 1H, 1-H (Pr)], 4.20 [dd, 1H, J = 6 and 15 Hz, CH_2 (Bn-ureido)], 4.28 [d, 2H, J = 6 CH_2 (Bn)], 4.32 [m, 1H, α-H (Orn)], 4.41 [dd, 1H, J = 6 and 15 Hz, CH_2 (Bn-

ureido)], 4.60 [dd, 1H, $J = 5$ and 15 Hz, CH_2 (Bn)], 6.94 and 7.11 (2bs, 2H, NHCONH), 7.18–7.30 (m, 15H, aromatics), 8.09 [bs, 3H, δ – NH_3^+ (Orn)], 9.19 and 9.86 [2bs, 2H, α – NH_2^+ (Orn)], 9.54 (bs, 1H, *NH*-Bn); ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.3 (CH_2, C_γ), 27.9 (CH_2, C_β), 28.8 (CH_2, C_3), 35.4 (CH_2, C_δ), 38.6 (CH_2, Bn), 43.4 [CH_2, (Bn-ureido)], 43.7 (CH_2, C_1), 58.5 (CH, C_2), 60.6 (CH, C_α), 127.4–140.9 (15CH and 3C, aromatics), 160.5 (HNCONH), 167.9 (CONH); ES-MS *m/z* [M + 1]$^+$ calcd for $C_{29}H_{37}N_5O_2$, 488.29; found, 488.38 (100%).

4.5.10. N_α-[(2R)-1-(3-(2,4-Dichlorophenyl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide dihydrochloride [(**R**)-**19b**]

Amorphous solid (62 mg, 100%); HPLC t_R 14.85 min; $[\alpha]_D^{20}$ +9.8 (*c* 0.4, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.37–1.49 [m, 2H, γ-H (Lys)], 1.53–1.71 [m, 2H, δ-H (Lys)], 1.82–2.05 [m, 2H, β-H (Lys)], 2.80 [m, 2H, ε-H (Lys)], 2.91 [m, 1H, 3-H (Pr)], 3.23 [m, 1H, 2-H (Pr)], 3.27–3.40 [m, 3H, 1-H and 3-H (Pr)], 3.26 [m, 1H, α-H (Lys)], 3.26 [dd, 1H, $J = 6$ and 15 Hz, CH_2 (Bn)], 4.52 [dd, 1H, $J = 5$ and 15 Hz, CH_2 (Bn)], 7.18–7.44 (m, 13H, aromatics), 7.59 (bs, 2H, HNCONH), 7.94 [bs, 3H, δ – NH_3^+ (Lys)], 8.99 and 9.67 [2bs, 2H, α – NH_2^+ (Lys)], 9.43 (bs, 1H, *NH*-Bn); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.3 (CH_2, C_γ), 26.5 (CH_2, C_δ), 29.4 (CH_2, C_β), 32.0 (CH_2, C_3), 37.5 (CH_2, C_ε), 38.3 (CH_2, Bn), 42.6 (CH_2, C_1), 57.2 (CH, C_2), 58.8 (CH, C_α), 116.9–140.2 (13CH and 5C, aromatics), 155.9 (HNCONH), 167.1 (CONH); ES-MS *m/z* [M + 1]$^+$ calcd for $C_{29}H_{35}Cl_2N_5O_2$, 556.22; found, 556.63 (100%), 558.52 (64%) [M + 3]$^+$, 560.42 (10%) [M + 5]$^+$.

4.5.11. N_α-[(2S)-1-(3-(2,4-Dichlorophenyl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide dihydrochloride [(**S**)-**19b**]

Amorphous solid (63 mg, 100%); HPLC t_R 14.09 min; $[\alpha]_D^{20}$ +12.3 (*c* 1.1, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.17–1.42 [m, 2H, γ-H (Lys)], 1.46–1.681 [m, 2H, δ-H (Lys)], 1.68–1.98 [m, 2H, β-H (Lys)], 2.61–2.95 [m, 2H, 3-H (Pr)], 2.71 [m, 2H, ε-H (Lys)], 2.96–3.19 [m, 2H, 1-H (Pr)], 3.23 [m, 1H, 2-H (Pr)], 4.3 [m, 1H, α-H (Lys)], 4.36 [d, 2H, $J = 5$ Hz, CH_2 (Bn)], 7.11–7.39 (m, 15H, aromatics and HNCONH), 7.92 [bs, 3H, δ – NH_3^+ (Lys)], 9.16 [bs, 2H, α – NH_2^+ (Lys)], 9.41 (bs, 1H, *NH*-Bn); ^{13}C NMR (100 MHz, DMSO-d_6) δ 21.9 (CH_2, C_γ), 26.9 (CH_2, C_δ), 29.5 (CH_2, C_β), 35.0 (CH_2, C_3), 38.3 (CH_2, Bn), 38.8 (CH_2, C_ε), 43.1 (CH_2, C_1), 58.5 (CH, C_2), 59.0 (CH, C_α), 116.5–141.5 (13CH and 5C, aromatics), 156.2 (HNCONH), 167.9 (CONH); ES-MS *m/z* [M + 1]$^+$ calcd for $C_{29}H_{35}Cl_2N_5O_2$, 556.22; found, 556.63 (100%), 558.52 (64%) [M + 3]$^+$, 560.42 (10%) [M + 5]$^+$.

4.5.12. N_α-[(2R)-1-(3-(4-Methoxyphenethyl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide dihydrochloride [(**R**)-**20b**]

Amorphous solid (61 mg, 100%); HPLC t_R 13.46 min; $[\alpha]_D^{20}$ +15.6 (*c* 0.8, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.27–1.39 [m, 2H, γ-H (Lys)], 1.39–1.64 [m, 2H, δ-H (Lys)], 1.74–1.95 [m, 2H, β-H (Lys)], 2.68–2.79 [m, 1H, 3-H (Pr)], 2.63 [t, 2H, $J = 7$ Hz, 2-H (ethyl)], 2.71 [m, 2H, ε-H (Lys)], 3.03–3.25 [m, 6H, 1-H, 2-H, 3-H (Pr) and 1-H (ethyl)], 3.69 (s, 3H, OMe), 4.09 [m, 1H, α-H (Lys)], 4.28 [dd, 1H, $J = 6$ and 15 Hz, CH_2 (Bn)], 4.47 [dd, 1H, $J = 5$ and 15 Hz, CH_2 (Bn)], 6.37 and 6.50 (2bs, 2H, HNCONH), 6.69–7.38 (m, 14H, aromatics), 7.80 [bs, 3H, δ – NH_3^+ (Lys)], 9.25 [bs, 2H, α – NH_2^+ (Lys)], 9.45 (bs, 1H, *NH*-Bn); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.6 (CH_2, C_γ), 27.2 (CH_2, C_δ), 30.5 (CH_2, C_β), 34.3 (CH_2, C_3), 35.4 [CH_2, C_2 (ethyl)], 38.0 [CH_2, C_ε and C_1 (ethyl)], 38.0 (CH_2, C_ε), 38.8 (CH_2, Bn), 41.9 (CH_2, C_1), 55.4 (CH, C_2), 55.4 (CH_3, OMe), 56.8 (CH, C_α), 114.0–158.0 (14CH and 4C, aromatics), 161.5 (HNCONH), 167.9 (CONH); ES-MS *m/z* [M + 1]$^+$ calcd for $C_{32}H_{43}N_5O_3$, 546.34; found, 546.62 (100%).

4.5.13. N_α-[(2S)-1-(3-(4-Methoxyphenethyl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide dihydrochloride [(**S**)-**20b**]

Amorphous solid (62 mg, 100%); HPLC t_R 13.52 min; $[\alpha]_D^{20}$ +19.7 (*c* 0.7, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.35 [m, 2H, γ-H (Lys)], 1.594 [m, 2H, δ-H (Lys)], 1.83 [m, 2H, β-H (Lys)], 2.62 [m, 2H, ε-H (Lys)], 2.71 [m, 2H, 2-H (ethyl)], 2.96 [m, 1H, 3-H (Pr)], 3.03 [m, 3H, 1-H and 3-H (Pr)], 3.15 [d, 2H, $J = 7$ Hz, 1-H (ethyl)], 3.20 [m, 1H, 2-H (Pr)], 3.69 (s, 3H, OMe), 4.21 [m, 1H, α-H (Lys)], 4.36 [m, 2H, CH_2 (Bn)], 6.57 and 6.76 (2bs, 2H, HNCONH), 7.14–7.3 (m, 14H, aromatics), 8.03 [bs, 3H, δ – NH_3^+ (Lys)], 9.00 and 9.92 [2bs, 2H, α – NH_2^+ (Lys)], 9.45 (bs, 1H, *NH*-Bn); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.3 (CH_2, C_γ), 26.3 (CH_2, C_δ), 29.6 (CH_2, C_β), 34.7 [CH_2, C_2 (ethyl)], 34.9 (CH_2, C_3), 38.1 [CH_2, C_1 (ethyl)], 38.7 (CH_2, C_ε), 40.1 (CH_2, Bn), 42.6 (CH_2, C_1), 54.9 (CH_3, OMe), 58.4 (CH, C_2), 60.0 (CH, C_α), 113.8–157.6 (14CH and 4C, aromatics), 159.8 (HNCONH), 167.4 (CONH); ES-MS *m/z* [M + 1]$^+$ calcd for $C_{32}H_{43}N_5O_3$, 546.34; found, 546.62 (100%).

4.5.14. N_α-[(2R)-1-((3-(4-Fluorophenethyl)ureido)-3-phenyl) propan-2-yl]-lysine benzyl amide dihydrochloride [(**R**)-**21b**]

Amorphous solid (60 mg, 100%); HPLC t_R 13.65 min; $[\alpha]_D^{20}$ +15.2 (*c* 0.8, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.30–1.41 [m, 2H, γ-H (Lys)], 1.50–1.67 [m, 2H, δ-H (Lys)], 1.75–1.98 [m, 2H, β-H (Lys)], 2.68 [t, 2H, $J = 7$ Hz, 2-H (ethyl)], 2.71 [m, 2H, ε-H (Lys)], 2.78 [m, 1H, 3-H (Pr)], 3.04 [m, 1H, 2-H (Pr)], 3.08–3.26 [m, 5H, 1-H, 3-H (Pr), and 1-H (ethyl)], 4.21 [m, 1H, α-H (Lys)], 4.28 [dd, 1H, $J = 6.5$ and 15 Hz, CH_2 (Bn)], 4.46 [dd, 1H, $J = 5$ and 15 Hz, CH_2 (Bn)], 6.43 and 6.59 (2bs, 2H, HNCONH), 7.02–7.36 (m, 14H, aromatics), 7.95 [bs, 3H, δ – NH_3^+ (Lys)], 9.42 and 9.84 [2bs, 2H, α – NH_2^+ (Lys)], 9.48 (bs, 1H, *NH*-Bn); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.6 (CH_2, C_γ), 26.8 (CH_2, C_δ), 30.0 (CH_2, C_β), 33.9 (CH_2, C_3), 35.3 [CH_2, C_2 (ethyl)], 38.2 [CH_2, C_1 (ethyl)], 38.7 (CH_2, C_ε), 41.7 (CH_2, Bn), 42.9 (CH_2, C_1), 57.6 (CH, C_2), 61.2 (CH, C_α), 115.2–160.1 (14CH and 4C, aromatics), 162.2 (HNCONH), 167.5 (CONH); ES-MS *m/z* [M + 1]$^+$ calcd for $C_{31}H_{40}FN_5O_2$, 534.32; found, 534.65 (100%).

4.5.15. N_α-[(2S)-1-((3-(4-Fluorophenethyl)ureido)-3-phenyl) propan-2-yl]-lysine benzyl amide dihydrochloride [(**S**)-**21b**]

Amorphous solid (59 mg, 100%); HPLC t_R 13.69 min; $[\alpha]_D^{20}$ +21.5 (*c* 1.3, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.36 [m, 2H, γ-H (Lys)], 1.59 [m, 2H, δ-H (Lys)], 1.84 [m, 2H, β-H (Lys)], 2.68 [t, 2H, $J = 7$ Hz, 2-H (ethyl)], 2.72 [m, 2H, ε-H (Lys)], 2.78 [m, 1H, 3-H (Pr)], 2.97 [m, 1H, 1-H (Pr)], 3.04 [m, 1H, 3-H (Pr)], 3.15 [m, 1H, 2-H (Pr)], 3.18 [m, 1H, 1-H (Pr)], 3.22 [t, 2H, $J = 7$ Hz, 1-H (ethyl)], 4.23 [m, 1H, α-H (Lys)], 4.36 [d, 2H, $J = 6$ Hz, CH_2 (Bn)], 6.63 and 6.82 (2bs, 2H, HNCONH), 7.10–7.34 (m, 14H, aromatics), 8.07 [bs, 3H, δ – NH_3^+ (Lys)], 9.02 and 9.91 [2bs, 2H, α – NH_2^+ (Lys)], 9.48 (bs, 1H, *NH*-Bn); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.1 (CH_2, C_γ), 26.7 (CH_2, C_δ), 31.8.0 (CH_2, C_β), 35.2 (CH_2, C_3), 35.5 [CH_2, C_2 (ethyl)], 38.7 [CH_2, C_1 (ethyl)], 39.1 (CH_2, C_ε), 40.6 (CH_2, Bn), 43.0 (CH_2, C_1), 58.9 (CH, C_2), 60.4 (CH, C_α), 115.4–160.3 (14CH and 4C, aromatics), 162.2 (HNCONH), 167.9 (CONH); ES-MS *m/z* [M + 1]$^+$ calcd for $C_{31}H_{40}FN_5O_2$, 534.32; found, 534.65 (100%).

4.6. Synthesis of the indazole-containing protected ureas (**R**)- and (**S**)-**24b**

Diethyl amine (0.22 mL, 2.1 mmol) was added to a solution of the corresponding Fmoc-protected amine (**R**)- and (**S**)-**12b** (0.21 mmol) and the reaction mixture was stirred at rt for 2 h. Then, the solvent was evaporated to dryness and the residue was dissolved in EtOAc (50 mL). The solution was successively washed with H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4, and evaporated to dryness to give the deprotected amines (**R**)- and (**S**)-**3b**, which were reserved. Apart, propylene oxide (118 μL, 1.68 mmol) was added to a 0 °C cooled solution of 1-(2,6-dichlorobenzyl)-3-(pyrrolidin-1-ylmethyl)-1*H*-indazole-6-amine [50] (**22**) (79 mg, 0.21 mmol) in dry THF (5 mL). Then, a solution of bis(trichloromethyl)carbonate (20 mg, 0.07 mmol) in dry THF (1 mL) was added dropwise and stirring was maintained at 0 °C for 15 min. Afterwards, the mixture was added dropwise to a 0 °C cooled solution of (**R**)- and (**S**)-**3b** (98 mg, 0.21 mmol) in dry THF (5 mL)

and stirred for 1 h. Then, the reaction mixture was diluted with EtOAc (50 mL), washed successively with H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by reversed phase chromatography, using 10–100% CH_3CN gradient in 0.05% TFA solution in H_2O as mobile phase.

*4.6.1. N_ε-Boc-N_α-[(2R)-1-(3-(1-(2,6-dichlorobenzyl)-3-(pyrrolidin-1-ylmethyl)-1H-indazol-6-yl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide [(**R**)-**24b**]*

Foam (36 mg, 20%); HPLC t_R 16.33 min; HPLC-MS t_R 3.26 min; $[\alpha]_D^{20}$ +5.6 (*c* 1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02–1.46 [m, 4H, γ- and δ-H (Lys)], 1.31 (s, 9H, Boc), 1.69, 1.85, 3.02, 3.45 (4m, 8H, pyrrolidine), 1.75–1.94 [m, 2H, β-H (Lys)], 2.74–2.92 [m, 2H, ε-H (Lys)], 2.74–3.07 [m, 2H, 3-H (Pr)], 3.27 [m, 1H, 2-H (Pr)], 3.21–3.43 [m, 2H, 1-H (Pr)], 3.93 [bs, 1H, α-H (Lys)], 4.29 [d, J = 7 Hz, 2H, CH_2 (Bn)], 4.36 (s, 2H, *CH_2*–pyrrolidine), 4.64 (s, 1H, *NH*-Boc), 5.52 (s, 2H, *CH_2*-diClPh), 6.94 [d, J = 7 Hz, 1H, 5-H (indazole)], 7.36–7.00 (m, 13H, Ph), 7.41 [d, J = 7 Hz, 1H, 4-H (indazole)], 7.87 [s, 1H, 7-H (indazole)], 8.12 (s, 1H, *NH*-Bn), 8.72 and 11.8 (2bs, 2H, HNCONH); ^{13}C NMR (100 MHz, $CDCl_3$) 21.7 (CH_2, C_γ), 23.4 (CH_2, pyrrolidine), 28.3 (CH_3, Boc), 29.1 (CH_2, C_δ), 29.5 (CH_2, C_β), 34.9 (CH_2, C_3), 40.8 (CH_2, C_1), 43.8 (CH_2, Bn), 47.7 (*CH_2*-diClPh), 48.1, 52.3 (CH_2, pyrrolidine and *CH_2*–pyrrolidine), 59.3 (CH, C_α), 60.2 (CH, C_2), 79.5 (C, Boc), 98.8 [C_7 (indazole)], 116.3[C_5 (indazole)], 119.2 [C_4 (indazole)], 127.6–129.2 (CH, Ph), 130.1 (C, Ph), 131.2 (C, diClPh), 133.7 [C_5 (indazole)], 136.8 (C, Bn), 137.3 (2C, diClPh), 138.8 [C_6 (indazole)], 141.6 [C_{7a} (indazole)], 157.4 2 (HNCONH), 162.2 (CO, Boc), 167.2 (CONH); ES-MS m/z $[M + 1]^+$ calcd. for $C_{47}H_{58}Cl_2N_8O_4$, 869.40; found, 435.29 (100%) $[(M + 2)/2]^+$, 869.49 (6%) $[M + 1]^+$. Anal. calcd. for $C_{47}H_{58}Cl_2N_8O_4$: C, 64.89; H, 6.72; N, 12.88. Found: C, 64.95; H, 6.82; N, 12.95.

*4.6.2. N_ε-Boc-N_α-[(2S)-1-(3-(1-(2,6-dichlorobenzyl)-3-(pyrrolidin-1-ylmethyl)-1H-indazol-6-yl)ureido)-3-phenylpropan-2-yl]-lysine benzyl amide [(**S**)-**24b**]*

Foam (155 mg, 85%); HPLC t_R 16.51 min; HPLC-MS t_R 3.26 min; $[\alpha]_D^{20}$ +8.4 (*c* 1, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.08–1.42 [m, 4H, γ- and δ-H (Lys)], 1.29 (s, 9H, Boc), 1.70, 1.87, 2.98, 3.45 (4m, 8H, pyrrolidine), 1.76–1.83 [m, 2H, β-H (Lys)], 2.72–2.86 [m, 2H, ε-H (Lys)], 2.74–3.07 [m, 2H, 3-H (Pr)], 3.23 [m, 1H, 2-H (Pr)], 3.18–3.31 [m, 1H, 1-H (Pr)], 3.55 [m, 1H, 1-H (Pr)], 4.25 [bs, 1H, α-H (Lys)], 4.30 [dd, J = 5 and 15 Hz, 2H, CH_2 (Bn)], 4.36 (s, 2H, *CH_2*–pyrrolidine), 4.70 (s, 1H, *NH*-Boc), 5.50 (s, 2H, *CH_2*-diClPh), 6.99 [d, J = 7 Hz, 1H, 5-H (indazole)], 7.09–7.34 (m, 13H, Ph), 7.43 [d, J = 7 Hz, 1H, 4-H (indazole)], 7.80 [s, 1H, 7-H (indazole)], 9.18 (s, 1H, *NH*-Bn), 8.92 and 11.78 (2bs, 2H, HNCONH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.9 (CH_2, C_γ), 23.6 (CH_2, pyrrolidine), 28.5 (CH_3, Boc), 29.3 (CH_2, C_δ), 30.7 (CH_2, C_β), 30.7 (CH_2, C_β), 36.6 (CH_2, C_3), 39.7 (CH_2, C_ε), 40.3 (CH_2, C_1), 44.2 (CH_2, Bn), 48.0 (*CH_2*-diClPh), 48.6 (*CH_2*–pyrrolidine), 52.8 (CH_2, pyrrolidine), 60.6 (CH, C_α), 61.5 (CH, C_2), 79.2 (C, Boc), 98.8 [C_7 (indazole)], 116.4 [C_5 (indazole)], 119.3 [C_4 (indazole)], 119.8 [C_{3a} (indazole)], 167.6 (CONH), 127.8–129.5 (CH, Ph), 130.5 (C, Ph), 131.6 (C, diClPh), 133.9 [C_5 (indazole)], 134.3 (C, Bn), 137.0 (2C, diClPh), 138.8 [C_6 (indazole)], 141.7 [C_{7a} (indazole)], 158.5 (C, HNCONH), 162.1 (CO, Boc), 167.6 (CONH); ES-MS m/z $[M + 1]^+$ calcd. for $C_{47}H_{58}Cl_2N_8O_4$, 869.40; found, 435.29 (100%) $[(M + 2)/2]^+$, 869.49 (5%) $[M + 1]^+$; Anal. calcd. for $C_{47}H_{58}Cl_2N_8O_4$: C, 64.89; H, 6.72; N, 12.88. Found: C, 65.02; H, 6.85; N, 12.92.

*4.6.3. N-Boc deprotection of (**S**)-**24b**. Synthesis of N_α-[(2S)-1-(3-(1-(2,6-dichlorobenzyl)-3-(pyrrolidin-1-ylmethyl)-1H-indazol-6-yl) ureido)-3-phenylpropan-2-yl]-lysine benzyl amide trihydrochloride [(**S**)-**25b**]*

The protected urea (**S**)-**24b** (130 mg, 0.15 mmol) was dissolved in a 3 M solution of HCl in EtOAc (2 mL) and stirred at rt for 2 h. Then, the solvent was evaporated under reduced pressure, the residue was dissolved in H_2O (3 mL) and the solution was lyophilized to give the urea (**S**)-**25b** (131 mg, 100%). HPLC t_R 13.11 min; HPLC-MS t_R 2.73 min; $[\alpha]_D^{20}$ +1.6 (*c* 1.2, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 1.32 [m, 2H, γ-H (Lys)], 1.53 [m, 2H, δ-H (Lys)], 1.74 [m, 2H, β-H (Lys)], 1.77–1.95 [m, 4H, $2CH_2$ (pyrrolidine)], 2.66 [m, 2H, ε-H (Lys)], 2.84 [dd, 1H, J = 10 and 13 Hz, 3-H (Pr)], 3.04 [m, 4H, $2CH_2$ (pyrrolidine)], 3.08–3.16 [m, 1H, 3-H (Pr)], 3.12 [m, 1H, 2-H (Pr)], 3.19–3.59 [m, 2H, 1-H (Pr)], 4.14 [m, 1H, α-H (Lys)], 4.32 [t, 2H, J = 7 Hz, CH_2 (Bn)], 4.54 (s, 2H, *CH_2*–pyrrolidine), 5.57 (s, 2H, *CH_2*-diClPh), 7.02–7.95 (m, 16H, aromatics), 8.01 and 9.66 (2s, 2H, HNCONH), 9.08 (s, 1H, *NH*-Bn), 9.43 and 10.58 [2bs, 2H, α – NH_2^+ (Lys)]. ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 21.4 (CH_2, C_γ), 22.6 ($2CH_2$, pyrrolidine), 26.4 (CH_2, C_δ), 29.7 (CH_2, C_β), 35.1 (CH_2, C_3), 38.2 (CH_2, C_ε), 38.3 (CH_2, C_1), 42.6 (CH_2, Bn), 47.4 (CH_2, diClPh), 47.7 (CH_2, *CH_2*–pyrrolidine), 52.7 ($2CH_2$, pyrrolidine), 58.2 (CH, C_α), 58.8 (CH, C_2), 98.4 [C_7 (indazole)], 114.6 [C_5 (indazole)], 117.8 [C_4 (indazole)], 120.6 [C_{3a} (indazole)], 127.1–131.4 (CH, aromatics), 135.5 (C, Bn), 136.1 (C, diClPh), 136.2 (C, Ph), 138.2 [C_5 (indazole)], 138.4 (2C, diClPh), 139.7 [C_6 (indazole)], 141.3 [C_{7a} (indazole)], 156.1 (HNCONH), 167.3 (CONH); ES-MS m/z $[M + 1]^+$ calcd for $C_{42}H_{50}Cl_2N_8O_2$, 769.34; found, 385.28 (60%) $[(M + 2)/2]^+$, 769.37 (6%) $[M + 1]^+$.

*4.7. General procedure for the synthesis of thioureas (**R**)- and (**S**)-**26a***

Diethyl amine (0.32 mL, 3 mmol) was added to a solution of the corresponding Fmoc-protected amine (**R**)- and (**S**)-**12a** (0.3 mmol) and the reaction mixture was stirred at rt for 2 h. Then, the solvent was evaporated to dryness and the residue was dissolved in EtOAc (50 mL). The solution was successively washed with H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4, and evaporated to dryness to give the deprotected amines (**R**)- and (**S**)-**3a**. These amines were dissolved in dry CH_2Cl_2 (10 mL) and the solution was cooled at 0 °C. Phenyl isothiocyanate (40.5 mg, 0.3 mmol) was added and the mixture was stirred for 2 h. Then, the solvent was evaporated and the residue was dissolved in EtOAc (50 mL). This solution was successively washed with H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using 20–100% EtOAc gradient in hexane as eluant to give the corresponding thiourea (**R**)- and (**S**)-**26a**.

*4.7.1. N_δ-Boc-N_α-[(2R)-3-phenyl-1-(3-phenylthioureido)propan-2-yl]-ornithine benzyl amide [(**R**)-**26a**]*

Foam (121 mg, 68%); HPLC t_R 18.42 min; $[\alpha]_D^{20}$ +3.0 (*c* 1, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38 (m, 9H, Boc), 1.10–1.65 [m, 4H, β-H and γ-H (Orn)], 2.71–2.91 [m, 3H, 3-H (Pr) and δ-H (Orn)], 2.97 [dd, 1H, J = 7.5 and 13 Hz, 3-H (Pr)], 3.09–3.30 [m, 2H, α-H (Orn) and 2-H (Pr)], 3.63 [d, 2H, J = 5 Hz, 1-H (Pr)], 4.21 [dd, J = 6.5 and 15 Hz, 1H, CH_2 (Bn)], 4.43 [dd, 1H, J = 5 and 15 Hz, CH_2 (Bn)], 4.53 (s, 1H, *NH*-Boc), 6.79 and 7.85 (2s, 2H, HnCSNH), 7.04–7.63 (m, 16H, aromatics and *NH*-Bn); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.9 (CH_2, C_γ), 28.3 (CH_3, Boc), 30.5 (CH_2, C_β), 39.4 (CH_2, C_δ), 43.2 (CH_2, Bn), 58.1 (CH, C_α), 59.7 (CH, C_2), 79.3 (C, Boc), 125.0–138.3 (15CH and 3 C, aromatics), 156.3 (CO, Boc), 174.9 (CONH), 181.2 (CS); ES-MS m/z $[M + 1]^+$ calcd. for $C_{33}H_{44}N_5O_3S$, 590.31; found, 590.51 (100%). Anal. calcd. for $C_{33}H_{43}N_5O_3S$: C, 67.20; H, 7.35; N, 11.87. Found: C, 67.24; H, 7.55; N, 11.93.

*4.7.2. N_δ-Boc-N_α-[(2S)-3-phenyl-1-(3-phenylthioureido)propan-2-yl]-ornithine benzyl amide [(**S**)-**26a**]*

Foam (127 mg, 72%); HPLC t_R 18.67 min; $[\alpha]_D^{20}$ +7.5 (*c* 1.1, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38 (s, 9H, Boc), 1.23–1.90 [m, 4H, β-H and γ-H (Orn)], 2.87–3.17 [m, 6H, 1- and 3-H (Pr), and δ-H (Orn)],

3.40 [m, 1H, 2-H (Pr)], 3.62 [m, 1H, α-H (Orn)], 4.10 [dd, $J = 7$ and 15 Hz, 1H, CH_2 (Bn)], 4.38 [dd, $J = 5$ and 15 Hz, 1H, CH_2 (Bn)], 4.71 (s, 1H, *NH*-Boc), 6.69 and 8.05 (2s, 2H, HNCSNH), 7.02–7.54 (m, 16H, aromatics and *NH*-Bn); ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.2 (CH_2, C_γ), 28.4 (CH_3, Boc), 29.3 (CH_2, C_β), 39.5 (CH_2, C_δ), 40.5 (CH_2, C_3), 43.1 (CH_2, Bn), 58.3 (CH, C_α), 59.3 (CH, C_2), 79.5 (C, Boc), 124.5–138.4 (15CH and 3C, aromatics), 156.4 (CO, Boc), 173.4 (CONH), 181.3 (CS); ES-MS m/z $[M + 1]^+$ calcd. for $C_{33}H_{43}N_5O_3S$, 590.31; found, 590.58 (100%). Anal. calcd. for $C_{33}H_{43}N_5O_3S$: C, 67.20; H, 7.35; N, 11.87. Found: C, 67.15; H, 7.45; N, 11.86.

4.8. N-Boc-deprotection of (**R**)- and (**S**)-**26a**. Synthesis of the thiourea hydrochlorides (**R**)- and (**S**)-**27a**

It was carried out by applying the above described methodology for *N*-Boc removal in the urea analogues.

4.8.1. N_α-[(2R)-3-Phenyl-1-(3-phenylthioureido)propan-2-yl]-ornithine benzyl amide dihydrochloride [(**R**)-**27a**]

Amorphous solid (56 mg, 100%); HPLC t_R 13.29 min; $[\alpha]_D^{20}$ +10.7 (*c* 1, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.50–1.76 [m, 2H, γ-H (Orn)], 1.78–2.06 [m, 2H, β-H (Orn)], 2.72–3.27 [m, 4H, 3-H (Pr) and δ-H (Orn)], 3.46–3.66 [m, 2H, 1-H (Pr)], 3.80 [m, 1H, 2-H (Pr)], 4.25 [dd, 1H, $J = 6$ and 15 Hz, CH_2(Bn)], 4.37 [m, 1H, α-H (Orn)], 4.53 [dd, 1H, $J = 5$ and 15 Hz, CH_2(Bn)], 7.10 (s, 1H, HNCSNH), 6.93–7.55 (m, 16H, aromatics and HNCSNH), 8.05 [bs, 3H, $\delta - NH_3^+$ (Orn)], 8.75 and 10.22 [2bs, 2H, $\alpha - NH_2^+$ (Orn)], 9.41 (s, 1H, *NH*-Bn); ^{13}C NMR (125 MHz, DMSO-d_6) δ 22.8 (CH_2, C_γ), 27.1 (CH_2, C_β), 28.2 (CH_2, C_3), 34.1 (CH_2,C_δ), 38.1 (CH_2, Bn), 42.7 (CH_2, C_1), 57.0 (CH, C_2), 58.3 (CH, C_α),117.7–139.9 (15CH and 3C, aromatics), 166.9 (CONH), 181.0 (CS); ES-MS m/z $[M + 1]^+$ calcd. for $C_{28}H_{35}N_5OS$, 490.26; found 246.13(100%) $[(M + 2)/2]^+$, 490.48 (15%) $[M + 1]^+$.

4.8.2. N_α-[(2S)-3-Phenyl-1-(3-phenylthioureido)propan-2-yl]-ornithine benzyl amide dihydrochloride [(**S**)-**27a**]

Amorphous solid (54 mg, 100%); HPLC t_R 13.40 min; $[\alpha]_D^{20}$ +2.9 (*c* 0.7, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.51–2.06 [m, 4H, β- and γ-H (Orn)], 2.73–3.22 [m, 5H, 2- and 3-H (Pr), and δ-H (Orn)], 3.52–3.60 [m, 2H, 1-H (Pr)], 4.29 [m, 1H, α-H (Orn)], 4.40 [d, 2H, $J = 6$ Hz, CH_2 (Bn)], 7.10 (s, 1H, HNCSNH), 6.69–7.71 (m, 15H, aromatics and HNCSNH), 7.94 [bs, 3H, $\delta - NH_3^+$ (Orn)], 9.42 (s, 1H, *NH*-Bn), 9.19 and 9.54 [2bs, 2H, $\alpha - NH_2^+$ (Orn)]; ^{13}C NMR (125 MHz, DMSO-d_6) δ 22.7 (CH_2, C_γ), 27.1 (CH_2, C_β), 31.6 (CH_2, C_3), 38.3 (CH_2,C_δ), 38.9 (CH_2, Bn), 42.8 (CH_2, C_1), 57.0 (CH, C_2), 58.8 (CH, C_α), 123.4–138.7 (15CH and 3C, aromatics), 166.9 (CONH), 181.7 (CS); ES-MS m/z $[M + 1]^+$ calcd. for $C_{28}H_{35}N_5OS$, 490.26; found 246.13 (100%) $[(M + 2)/2]^+$, 490.48 (15%) $[M + 1]^+$.

4.9. Platelet aggregation inhibition assay

Whole blood was obtained from human volunteers who were not taking any platelet altering drugs for two weeks prior to donation. Blood was collected by venous puncture into 2.7 mL vacutainer tubes containing 3.2% buffered sodium citrate. Blood was centrifuged at 250×*g* for 7 min to obtain platelet rich plasma (PRP). After removal of PRP, the blood was re-centrifuged at 900×*g* for 10 min to obtain platelet poor plasma (PPP). The PPP was used as a reference in the optical aggregation and as a diluent to achieve a final platelet concentration of 200.000 platelet/μL in PRP. Tests were performed in an optical aggregometer (Chrono-Log Model 440 Four Channel). Briefly, a 0.5 mL sample of diluted PRP was added to a glass cuvette and incubated with either vehicle (DMSO solution) or tested compound, at a 0.1 mg/mL concentration, for 5 min at 37 °C. At the beginning of each experiment, aggregation response to SFLLRN (30 μM) was evaluated and the maximum aggregation value at the end of 5 min was recorded. Aggregation response to SFLLRN plus compound was recorded and compared to control (SFLLRN/vehicle) to determine the % of inhibition. Each compound was tested twice and the results are the mean of the two assays.

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References

[1] E. Di Cera, Thrombin, Mol. Aspects Med. 29 (2008) 203–254.
[2] S.R. Macfarlane, M.J. Seatter, T. Kanke, G.D. Hunter, R. Plevin, Proteinase-activated receptors, Pharmacol. Rev. 53 (2001) 245–282.
[3] L. Martorell, J. Martínez-González, C. Rodríguez, M. Gentile, O. Calvayrac, L. Badimon, Thrombin and protease-activated receptors (PARs) in atherothrombosis, Thromb. Haemost. 99 (2008) 305–315.
[4] V.S. Ossovskaya, N.W. Bunnett, Protease-activated receptors: contribution to physiology and disease, Physiol. Rev. 84 (2004) 579–621.
[5] E. Sokolova, G. Reiser, Prothrombin/thrombin and the thrombin receptors PAR-1 and PAR-4 in the brain: localization, expression and participation in neurodegenerative diseases, Thromb. Haemost. 100 (2008) 576–581.
[6] W. Luo, Y. Wang, G. Reiser, The role of thrombin and thrombin receptors in the brain, in: M.E. Maragoudakis, N.E. Tsopanoglou (Eds.), Thrombin: Physiology and Disease, Springer, New York, 2009, pp. 133–159.
[7] Y. Hashimotodani, T. Ohno-Shosaku, M. Yamazaki, K. Sakimura, M. Kano, Neuronal protease-activated receptor 1 drives synaptic retrograde signaling mediated by the endocannabinoid 2-arachidonoylglycerol, J. Neurosci. 31 (2011) 3104–3109.
[8] M. Steinhoff, J. Buddenkotte, V. Shpacovitch, A. Rattenholl, C. Moormann, N. Vergnolle, T.A. Luger, M.D. Hollenberg, Proteinase-activated receptors: transducers of proteinase-mediated signaling in inflammation and immune response, Endocr. Rev. 26 (2005) 1–43.
[9] R. Ramachandran, M. El-Daly, M. Saifeddine, M.D. Hollenberg, Thrombin: to PAR or not to PAR, and the regulation of inflamation, in: M.E. Maragoudakis, N.E. Tsopanoglou (Eds.), Thrombin: Physiology and Disease, Springer, New York, 2009, pp. 19–46.
[10] C.N. Pagel, S.J. Song, L.H. Loh, E.M. Tudor, T.A. Murray-Rust, R.N. Pike, E.J. Mackie, Thrombin-stimulated growth factor and cytokine expression in osteoblasts is mediated by protease-activated receptor-1 and prostanoids, Bone 44 (2009) 813–821.
[11] P. Arora, T.K. Ricks, J. Trejo, Protease-activated receptor signalling, endocytic sorting and dysregulation in cancer, J. Cell. Sci. 120 (2007) 921–928.
[12] E. Camerer, Protease signaling in tumor progression, Thromb. Res. 120 (Suppl. 2) (2007) S75–S81.
[13] K.S. Borensztajn, C.A. Spek, Protease-activated receptors, apoptosis and tumor growth, Pathophysiol. Haemost. Thromb. 36 (2008) 137–147.
[14] B. Kobrinsky, S. Karpatkin, The role of thrombin in tumor biology, in: M.E. Maragoudakis, N.E. Tsopanoglou (Eds.), Thrombin: Physiology and Disease, Springer, New York, 2009, pp. 161–172.
[15] M.T. García-López, M. Gutiérrez-Rodríguez, R. Herranz, Thrombin-activated receptors: promising targets for cancer therapy? Curr. Med. Chem. 17 (2010) 109–128.
[16] S.R. Coughlin, Thrombin signalling and protease-activated receptors, Nature 407 (2000) 258–264.
[17] J. Trejo, Regulation of thrombin receptor signaling, in: M.E. Maragoudakis, N.E. Tsopanoglou (Eds.), Thrombin: Physiology and Disease, Springer, New York, 2009, pp. 47–61.
[18] R. Shah, Protease-activated receptors in cardiovascular health and diseases, Am. Heart J. 157 (2009) 253–262.
[19] C. Chen, B.E. Maryanoff, P. Andrade-Gordon, Thrombin receptor modulators: medicinal chemistry, biological evaluation, and clinical applications, in: M.E. Maragoudakis, N.E. Tsopanoglou (Eds.), Thrombin: Physiology and Disease, Springer, New York, 2009, pp. 205–236.
[20] C. Tellez, M. Bar-Eli, Role and regulation of the thrombin receptor (PAR-1) in human melanoma, Oncogene 22 (2003) 3130–3137.
[21] S. Granovsky-Grisaru, S. Zaidoun, D. Grisaru, Y. Yekel, D. Prus, U. Beller, R. Bar-Shavit, The pattern of protease activated receptor 1 (PAR1) expression in endometrial carcinoma, Gynecol. Oncol. 103 (2006) 802–806.
[22] X. Zhang, W. Wang, L.D. True, R.L. Vessella, T.K. Takayama, Protease-activated receptor-1 is upregulated in reactive stroma of primary prostate cancer and bone metastasis, Prostate 69 (2009) 727–736.
[23] C. Rudroff, S. Seibold, R. Kaufmann, C.C. Zetina, K. Reise, U. Schafer, A. Schneider, M. Brockmann, J. Scheele, E.A. Neugebauer, Expression of the thrombin receptor PAR-1 correlates with tumour cell differentiation of pancreatic adenocarcinoma in vitro, Clin. Exp. Metastasis 19 (2002) 181–189.

[24] S. Grisaru-Granovsky, Z. Salah, M. Maoz, D. Pruss, U. Beller, R. Bar-Shavit, Differential expression of protease activated receptor 1 (Par1) and pY397FAK in benign and malignant human ovarian tissue samples, Int. J. Cancer 113 (2005) 372–378.
[25] D. Darmoul, V. Gratio, H. Devaud, T. Lehy, M. Laburthe, Aberrant expression and activation of the thrombin receptor protease-activated receptor-1 induces cell proliferation and motility in human colon cancer cells, Am. J. Pathol. 162 (2003) 1503–1513.
[26] P. Ghio, S. Cappia, G. Selvaggi, S. Novello, P. Lausi, G. Zecchina, M. Papotti, P. Borasio, G.V. Scagliotti, Prognostic role of protease-activated receptors 1 and 4 in resected stage IB non-small-cell lung cancer, Clin. Lung Cancer 7 (2006) 395–400.
[27] X. Zhang, J.L. Hunt, D.P. Landsittel, S. Muller, K. Adler-Storthz, R.L. Ferris, D.M. Shin, Z.G. Chen, Correlation of protease-activated receptor-1 with differentiation markers in squamous cell carcinoma of the head and neck and its implication in lymph node metastasis, Clin. Cancer Res. 10 (2004) 8451–8459.
[28] C.S.B. Veiga, T.C. Carneiro-Lobo, C.J.B.P. Coelho, S.M.F. Carvalho, R.C. Maia, F.C. Vasconcelos, E. Abdelhay, A.L. Mencalha, A.F. Ferreira, F.A. Castro, R.Q. Monteiro, Increased expression of protease-activated receptor 1 (PAR-1) in human leukemias, Blood Cells Mol. Dis. 46 (2011) 230–234.
[29] N.E. Tsopanoglou, M.E. Maragoudakis, The role of thrombin in angiogenesis, in: M.E. Maragoudakis, N.E. Tsopanoglou (Eds.), Thrombin: Physiology and Disease, Springer, New York, 2009, pp. 93–113.
[30] S. Chackalamannil, Y. Xia, W.J. Greenlee, M. Clasby, D. Doller, H. Tsai, T. Asberom, M. Czarniecki, H.S. Ahn, G. Boykow, C. Foster, J. Agans-Fantuzzi, M. Bryant, J. Lau, M. Chintala, Discovery of potent orally active thrombin receptor (protease activated receptor 1) antagonists as novel antithrombotic agents, J. Med. Chem. 48 (2005) 5884–5887.
[31] S. Chackalamannil, Y. Wang, W.J. Greenlee, Z. Hu, Y. Xia, H.S. Ahn, G. Boykow, Y. Hsieh, J. Palamanda, J. Agans-Fantuzzi, S. Kurowski, M. Graziano, M. Chintala, Discovery of a novel, orally active himbacine-based thrombin receptor antagonist (SCH 530348) with potent antiplatelet activity, J. Med. Chem. 51 (2008) 3061–3064.
[32] Y. Shinohara, S. Goto, M. Doi, P. Jensen, Safety of the novel protease-activated receptor-1 antagonist vorapaxar in Japanese patients with a history of ischemic stroke, J. Stroke Cerebrovasc. Dis. (2010). http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2010.1009.1005.
[33] H.D. White, Oral antiplatelet therapy for atherothrombotic disease: current evidence and new directions, Am. Heart J. 161 (2011) 450–461.
[34] T.K. Vu, D.T. Hung, V.I. Wheaton, S.R. Coughlin, Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation, Cell 64 (1991) 1057–1068.
[35] B.D. Blackhart, L. Ruslim-Litrus, C.C. Lu, V.L. Alves, W. Teng, R.M. Scarborough, E.E. Reynolds, D. Oksenberg, Extracellular mutations of protease-activated receptor-1 result in differential activation by thrombin and thrombin receptor agonist peptide, Mol. Pharmacol. 58 (2000) 1178–1187.
[36] T.K. Vu, V.I. Wheaton, D.T. Hung, I. Charo, S.R. Coughlin, Domains specifying thrombin-receptor interaction, Nature 353 (1991) 674–677.
[37] Y.M. Ayala, A.M. Cantwell, T. Rose, L.A. Bush, D. Arosio, E. Di Cera, Molecular mapping of thrombin-receptor interactions, Proteins 45 (2001) 107–116.
[38] M.T. Nieman, A.H. Schmaier, Interaction of thrombin with PAR1 and PAR4 at the thrombin cleavage site, Biochemistry 46 (2007) 8603–8610.
[39] S.L. Jacques, M. LeMasurier, P.J. Sheridan, S.K. Seeley, A. Kuliopulos, Substrate-assisted catalysis of the PAR1 thrombin receptor. Enhancement of macromolecular association and cleavage, J. Biol. Chem. 275 (2000) 40671–44068.
[40] T. Myles, B.F. Le Bonniec, S.R. Stone, The dual role of thrombin's anion-binding exosite-I in the recognition and cleavage of the protease-activated receptor 1, Eur. J. Biochem. 268 (2001) 70–77.
[41] W.F. Bahou, J.L. Kutok, A. Wong, C.L. Potter, B.S. Coller, Identification of a novel thrombin receptor sequence required for activation-dependent responses, Blood 84 (1994) 4195–4202.
[42] T. Nanevicz, M. Ishii, L. Wang, M. Chen, J. Chen, C.W. Turck, F.E. Cohen, S.R. Coughlin, Mechanisms of thrombin receptor agonist specificity. Chimeric receptors and complementary mutations identify an agonist recognition site, J. Biol. Chem. 270 (1995) 21619–21625.
[43] P.S. Gandhi, Z. Chen, F.S. Mathews, E. Di Cera, Structural identification of the pathway of long-range communication in an allosteric enzyme, Proc. Natl. Acad. Sci. U S A 105 (2008) 1832–1837.
[44] P.S. Gandhi, Z. Chen, E. Di Cera, Crystal structure of thrombin bound to the uncleaved extracellular fragment of PAR1, J. Biol. Chem. 285 (2010) 15393–15398.
[45] S. Seeley, L. Covic, S.L. Jacques, J. Sudmeier, J.D. Baleja, A. Kuliopulos, Structural basis for thrombin activation of a protease-activated receptor: inhibition of intramolecular liganding, Chem. Biol. 10 (2003) 1033–1041.
[46] J.A. González-Vera, M.T. García-López, R. Herranz, Potential of amino acid-derived a-amino nitriles for generating molecular diversity, Mini Rev. Org. Chem. 5 (2008) 209–221.
[47] P. Ventosa-Andrés, M.T. García-López, R. Herranz, A study on the induction of stereoselectivity in the Strecker synthesis of basic amino acid-derived α-amino nitriles, Tetrahedron: Asymmetry 23 (2012) 1198–1205.
[48] S. Herrero, M.T. García-López, R. Herranz, Expedient one-pot synthesis of novel chiral 2-substituted 5-phenyl-1,4-benzodiazepine scaffolds from amino acid-derived amino nitriles, J. Org. Chem. 68 (2003) 4582–4585.
[49] P.J. Kocienski, Protecting Groups, third ed., Georg Thieme, Stuttgart, 2004.
[50] Á.;.M. Valdivielso, M.T. García-López, R. Herranz, Improved synthesis of the PAR-1 thrombin receptor antagonist RWJ-58259, ARKIVOC xvii (2008) 287–294.
[51] B.E. Maryanoff, H.C. Zhang, P. Andrade-Gordon, C.K. Derian, Discovery of potent peptide-mimetic antagonists for the human thrombin receptor, protease-activated receptor-1 (PAR-1), Curr. Med. Chem. Cardiovasc. Hematol. Agents 1 (2003) 13–36.