Highly functionalized 2-oxopiperazine-based peptidomimetics: An approach to PAR1 antagonists

Ángel M. Valdivielso,[a] Pilar Ventosa-Andrés,[a] Francisco Tato,[a] M. Ángeles Fernández-Ibañez,[a] Ioannis Pappos,[b] Nikos E. Tsopanoglou[b] M. Teresa García-López,[a] Marta Gutiérrez-Rodríguez,[a] Rosario Herranz[a]*

[a]*Instituto de Química Médica (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain.*
[b]*Department of Pharmacology, Medical School, University of Patras, 26500 Rio-Patras, Greece.*

ABSTRACT

A series of pseudodipeptide-based chiral 1,3,4,5-tetrasubstituted-2-oxopiperazines has been designed and synthesized as potential PAR1 antagonists. These highly functionalized piperazines were synthesized from aromatic and basic amino acid derived Ψ[CH(CN)NH]pseudodipeptides through a four step pathway that involves reduction of the cyano group to build the 2-oxopiperazine ring, followed by selective functionalization at the N_4-, N_1-positions, and at the exocyclic moiety at position C_5. This regioselective functionalization required the fine tuning of reaction conditions. All new compounds were screened as inhibitors of human platelet aggregation induced by the PAR1 agonist SFLLRN and as cytotoxic agents in human cancer cell lines. Some of the compounds displayed moderate PAR1 antagonist activity, while, others were cytotoxic at μM concentration. No correlation was observed between both types of activities.

Keywords: PAR1 antagonists; Peptidomimetics; 2-Oxopiperazines; α-Amino nitriles; Platelet antiaggregant activity; Cytotoxicity.

1. **Introduction**

Thrombin induces multiple effects on a variety of cells, such as: platelets [1-4], endothelial and smooth muscle cells [1, 4, 5], neurons and astrocytes in the nervous system [1, 4-10], immune and inflammatory cells [4, 11, 12], and tumor cells [13-18]. These effects are mediated by the activation of the protease-activated receptor 1 (PAR1) [19]. This activation requires the thrombin-catalyzed cleavage of the N-terminal extracellular domain of PAR1 at the Arg^{41}/Ser^{42} peptide bond, which unveils the recognition sequence SFLLRN that acts as a tethered activation ligand.

*Corresponding autor: Fax: +34 915644853; Tel: +34 912587537; E-mail: rosario@iqm.csic.es

As 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, PAR1 antagonists have been searched almost exclusively in relation to the cardiovascular system up to now [2, 3, 20]. 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 [17, 21-28]. Based on these facts, this receptor is starting to be also considered a promising target for cancer therapy [19].

The first potent PAR1 antagonists were SFLLRN-based peptidomimetic ureas, represented by the optimized antagonist RWJ-58259 (Figure 1) [29], where an indazole ring is used as scaffold to assemble three key pharmacophoric groups (an aromatic and two amines). Later, several laboratories have reported a few series of antagonists obtained from HTS of diverse libraries of non-peptide small molecules, followed by optimization [20, 30], such SCH-530348 (named vorapaxar, Figure 1) [31], currently in Phase III clinical trials [32-34].

Mutagenesis, X-ray, and NMR studies 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 thrombin/PAR1 affinity [35-46]. These studies have also indicated 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. This knowledge prompted us to initiate a project directed to the search of new PAR1 antagonists based on the 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. To this aim, we planned the synthesis of diverse small directed libraries of different scaffolds able to assemble, at least, one or two aromatic groups and one or two basic groups at variable distances and orientations. As a first result of this project, we have recently reported a library of ureas and thioureas of general formula **A**. Some of these derivatives showed moderate antiaggregant activity in a screen of inhibitors of human platelet aggregation induced by the PAR1 agonist SFLLRN [47].

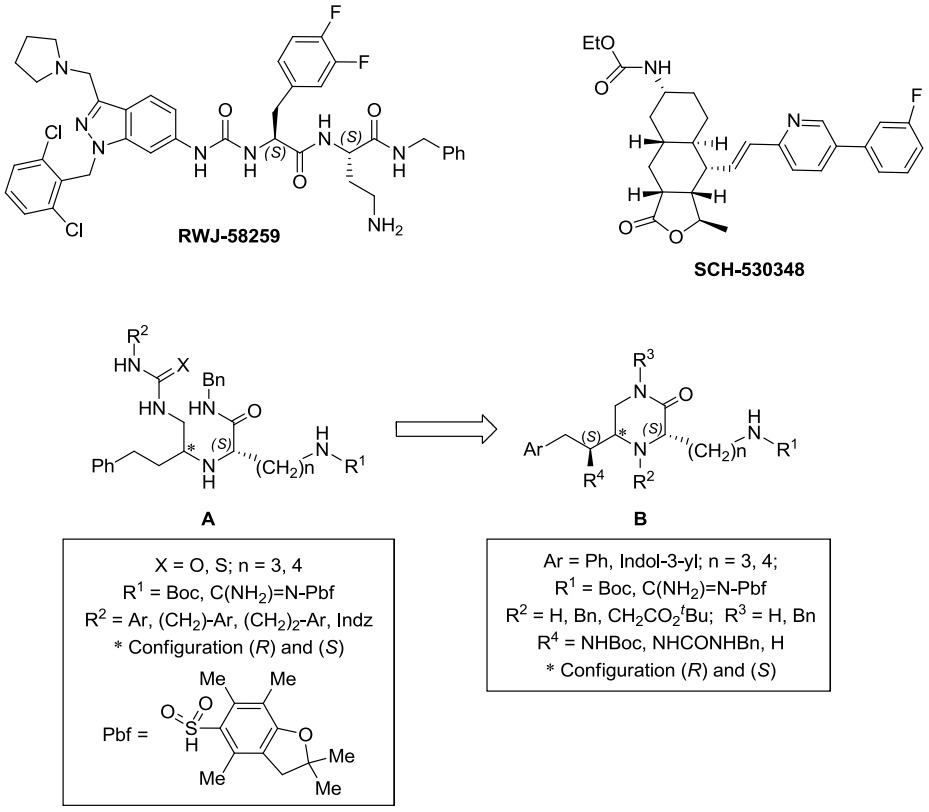


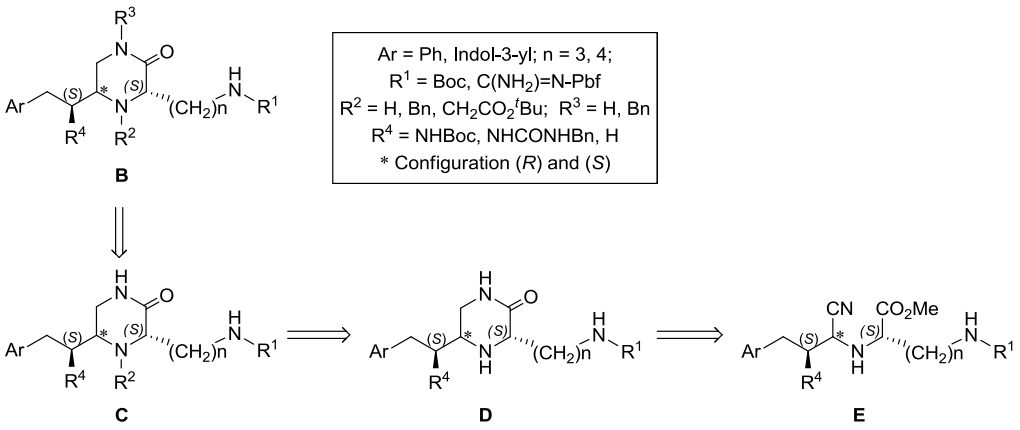
Fig. 1. Selected PAR1 antagonists and proposed 2-oxopiperazine derivatives **B.**

We now describe the synthesis and biological evaluation of a library of pseudodipeptide-based 2-oxopiperazine derivatives of general formula **B**, which could be considered as conformational restricted analogues of the urea derivatives **A**. The 2-oxopiperazine skeleton was selected as central core taking into account our synthetic experience in this heterocycle [48, 49] and that the piperazine ring is included among privileged scaffolds in medicinal chemistry. Actually, there are 165 drug entries for this heterocycle in the DrugBank database [50]. As in the series of urea derivatives **A** the side chain protection of the basic amino acid was necessary for their biological activity, the 2-oxopiperazine derivatives **B** were designed maintaining this protection. These piperazine derivatives have been screened as human PAR1 antagonists in a platelet aggregation assay and as cytotoxic agents in human cancer cell lines.

2. **Results and discussion**

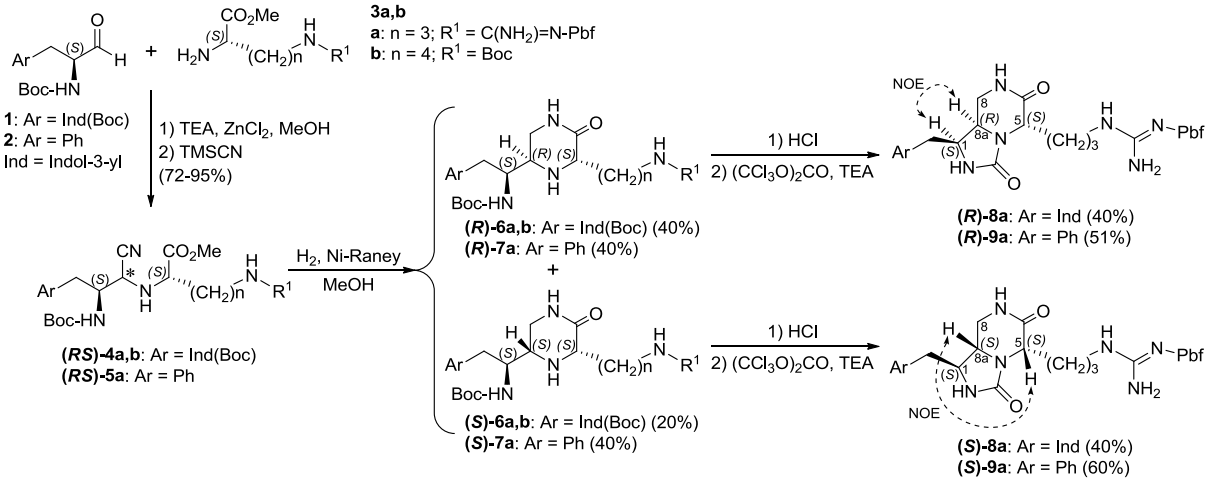
2.1. Synthesis

As shown in the retrosynthetic Scheme 1, a molecular diversity generation strategy in four steps was devised for the synthesis of the 2-oxopiperazine derivatives **B**, which started from the aromatic and basic amino acid derived Ψ[CH(CN)NH]pseudodipeptides **E**. This scheme consist of reduction of the cyano group, with subsequent *in situ* lactamization to give the 2-oxopiperazines **D**, followed by selective functionalization at the N_4 (**C**) and N_1 positions, and at the exocyclic moiety at position C_5 (R^4). Phenylalanine and tryptophan were selected as aromatic amino acids and lysine and arginine as basic amino acids.



Scheme 1. Retrosynthesis of the 2-oxopiperazines **B**.

The starting pseudodipeptides **4a,b** and **5a** (Scheme 2) were obtained by applying our methodology for the synthesis of Ψ[CH(CN)NH]pseudodipeptides [51], which involves a modified Strecker reaction of the protected α-amino aldehydes Boc-Trp(Boc)-H (**1**) and Boc-Phe-H (**2**) with the methyl esters of the side chain protected basic α-amino acids **3a** and **3b** at -20 ºC for 1 h, followed by *in situ* reaction with TMSCN at 0 ºC for 24 h. In this way, **4a,b** and **5a** were obtained as (*RS*)-epimeric mixtures at the cyano-bearing stereocenter, which could not be chromatographically resolved. In the tryptophan derivatives **(*RS*)-4a,b**, the (*R*)/(*S*) epimer ratio was (1:2), while in the phenylalanine derivatives **(*RS*)-5a**, this ratio was (1:1). The Ni Raney catalyzed hydrogenation of these epimeric mixtures at room temperature and 1 atm of H_2 pressure, with *in situ* lactamization, led to the corresponding 2-oxopiperazines **(*RS*)-6a,b** and **-7a** in 60-80% overall yield, which were chromatographically resolved in the respective (*R*)- and (*S*)-epimers.

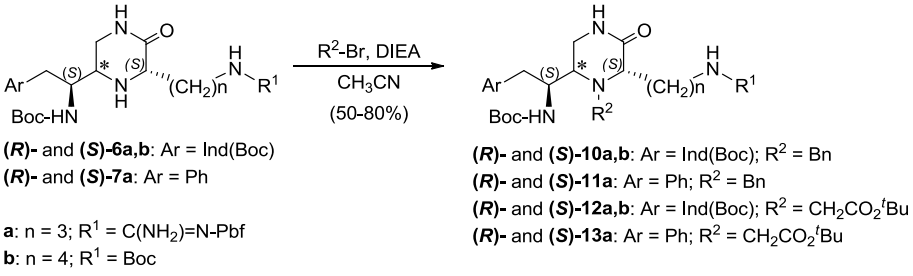


Scheme 2. Synthesis and configuration assignment of the 2-oxopiperazine derivatives **6a,b** and **7a**.

Signal overlapping of the 3-H and 5-H protons in the ^{1}H NMR spectra of **(*R*)-** and **(*S*)-6a,b** and **-7a** did not allow the NOE-based assignment of relative configuration at C_5 with respect to that at C_3. As the C_3 chiral center came from the starting basic amino acid, its configuration was known. Therefore, with the aim of ascertaining the C_5 configuration, the Arg derivatives **(*R*)-** and **(*S*)-6a** and **-7a** were transformed into the corresponding 3,6-dioxooctahydroimidazo[1,5-*a*]pyrazine derivatives **(*R*)-** and **(*S*)-8a** and **-9a** by Boc-removal, followed by reaction with bis(trichloromethyl)carbonate in the presence of TEA. As shown in Scheme 2, the NOE effect between 1-H and 8a-H protons observed in the NOESY 1D spectra of **(*R*)-8a** and **-9a** were indicative of a relative *cis* disposition and, therefore, a (*R*)-configuration at C_{8a}, and, therefore, a (*R*)-configuration at C_5 in **(*R*)-6a** and **-7a**. Similarly, the NOE effect between 5-H and 8a-H observed in the NOESY 1D spectra of **(*S*)-8a** and **-9a** were indicative of a *trans* relative disposition, and a (*S*)-configuration at C_{8a} in these compounds, and at C_5 in **(*S*)-6a** and **-7a**. The comparison of the ^{1}H NMR data of each pair of epimers of these Arg derived 2-oxopiperazines **6a** and **7a** showed that in the (*R*)-epimers both 6-H were anisochronous ($\Delta\delta \neq 0$ ppm), while in the (*S*) they were isochronous ($\Delta\delta = 0$ ppm). Furthermore, 5-H appeared at a lower field (0.05-0.07 ppm) in the (*R*)-epimers than in the (*S*). On the other hand, it was observed that C_3 and C_5 appeared 2.5-3.8 ppm at higher field in the (*R*)-epimers than in the (*S*) in the ^{13}C NMR spectra. These differences between epimers were used for the tentative configuration assignment in the lysine derived 2-oxopiperazines **6b**,

where the selective transformation into 3,6-dioxooctahydroimidazo[1,5-*a*]pyrazine derivatives without removal of the Boc group at the side chain was not possible.

The regioselective functionalization of the N_4 and N_1 positions of the 2-oxopiperazine ring of **6a,b** and **7b** was approached through alkylation reactions, by firstly applying the reaction conditions recently set up for related 2-oxopiperazine derivatives [49]. Thus, benzylation of **(*R*)-7a** was attempted by reaction with 1.1 equivalents of benzyl bromide in the presence of K_2CO_3 as base, under argon in CN_3CN solution at 60 ºC. After 24 h of reaction, the HPLC-MS analysis of the crude reaction showed 28% of the 4-benzyl derivative **(*R*)-11a** (Scheme 3) along with 72% of the starting compound **(*R*)-7a** (Table 1, entry 1). An increase of BnBr from the beginning of the reaction led to dirtier reactions. Therefore, to minimize side reactions an additional equivalent of BnBr and base was added each 24 h. In this way, after 3 days (Table 1, entry 3), 68% of **(*R*)-11a** was obtained. The starting material completely disappeared with 4.1 equivalents of reagents and 4 days of reaction, but, the reaction was significantly dirtier, to give only a 40% of the 4-benzyl derivative **(*R*)-11a** (entry 4). NaH, Cs_2CO_3 and diisiopropylethylamine (DIEA) were also tried as bases (Table 1, entries 5-10). Both NaH, Cs_2CO_3 led to very dirty reaction crudes. However, with DIEA the reactions were cleaner than with K_2CO_3, to provide 85% of **(*R*)-11a** by adding 4.1 equivalents of reagents (entry 10). These reaction conditions were also applied to the N_4-benzylation of the 2-oxopiperazines **(*S*)-7a** and **6a,b**, as well as for their N_4-alkylation with *tert*-butyl bromoacetate (Scheme 3). In general, (*R*)-epimers gave higher yields (65-80%) than their respective (*S*)-isomer (50-80%). In the ^{1}H NMR spectra the 6-H protons appeared as anisochronous, except for the (*R*)-epimer of the tryptophan derived N_4-*tert*-butoxycarbonylmethyl-2-oxopiperazines **(*R*)-12a,b**, where these protons appeared as isochronous, indicating high conformational flexibility.



Scheme 3. N_4-Alkylation of the 2-oxopiperazines **6a,b** and **7a**.

Table 1

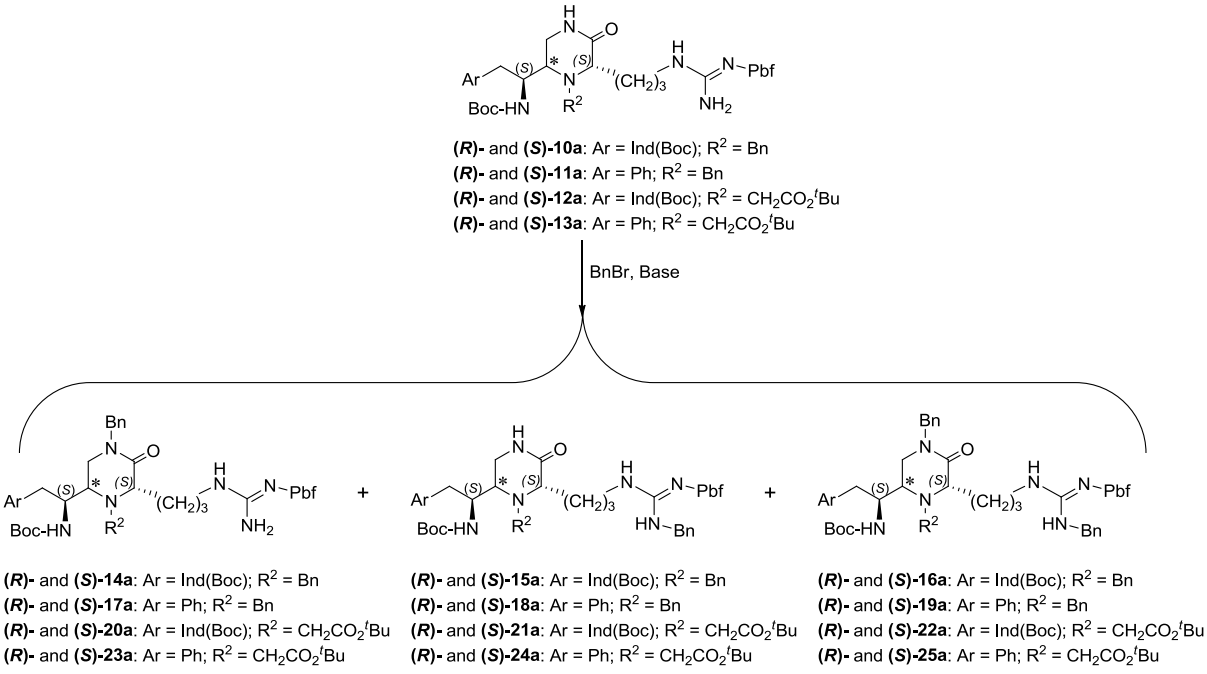
Optimization of the N_4-benzylation conditions for **(*R*)-7a**

Entry	Base	BnBr and base eq.	t (days)	Yield (%)[a]	
				(*R*)-7a	**(*R*)-11a**
1	K_2CO_3	1.1	1	72	28
2[b]	K_2CO_3	2.1	2	40	48
3[b]	K_2CO_3	3.1	3	10	68
4[b]	K_2CO_3	4.1	4	0	40
5	NaH	1.1	1	47	3
6	Cs_2CO_3	1.1	1	53	7
7	DIEA	1.1	1	70	30
8[b]	DIEA	2.1	2	41	59
9[b]	DIEA	3.1	3	26	74
10[b]	DIEA	4.1	4	0	85

[a] Determined by HPLC-MS analysis [Sunfire C_{18} (4.6×50 mm, 3.5 μm)]. [b]Addition of 1 equiv. each 24 h.

After the regiospecific alkylation at the N_4-position, benzylation at N_1 was studied by using the Phe and Arg derived 2-oxopiperazine **(*R*)-11a** for the setup of reaction conditions. Initially, this benzylation was attempted by applying previously described conditions developed for 2-oxopiperazine analogues [49], that is MW-activated reaction with BnBr in CH_3CN at 150 ºC, using Cs_2CO_3 as base (Table 2, entry 1). This attempt revealed the simultaneous alkylation at the guanidino group of arginine, in spite of being protected with the Pbf group. Thus, as shown in Scheme 4, the HPLC-MS analysis of the crude reaction mixture showed the formation of three products, of which that benzylated at N_1 and at the guanidino group **(*R*)-19a** (33%) was the major product. In view of this result, we decided to optimize the selective alkylation at N_1 by minimizing the alkylation at the guanidino group. Different solvents, bases, reagent stoichiometries, temperatures and reaction times were tried, whose results are summarized in Table 2. It was not possible to completely avoid the alkylation at the guanidino group and the best results were obtained when the benzylation was performed in (9:1) THF/DMF mixture at 0 ºC, adding 0.5 equivalents of BnBr and 1 equivalent of NaH in 1 h intervals during 3 h. In this way, the product of selective benzylation at N_1 **(*R*)-17a** was isolated in 58% yield, while, the product of dibenzylation at N_1 and at the

guanidino group **(*R*)-19a** was isolated in 20%. These reaction conditions were applied to the other N_4-sustituted-2-oxopiperazines **(*R*)-** and **(*S*)-**(**10a-13a**). Although the products resulting from monobenzylation at the guanidino group **(*R*)-** and **(*S*)-**(**15a**, **18a**, **21a** and **24a**) were identified in the HPLC-MS analysis of the crude reaction mixtures, they could not be isolated for their complete characterization.



Scheme 4. Benzylation of the arginine derived 4-substituted-2-oxopiperazines **10a-13a**.

Table 2

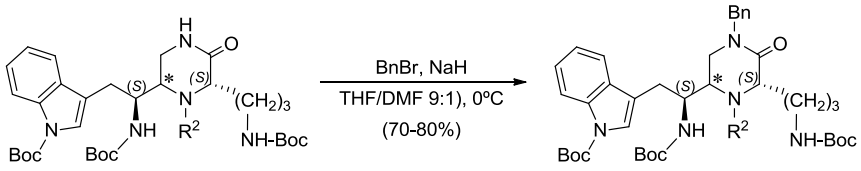
Optimization of the N_1-benzylation conditions for **(*R*)-11a**

Entry	Solvent	Base (eq.)	BnBr eq.	T (ºC)	t (h)	Yield (%)[a]		
						(*R*)-17a	**(*R*)-18a**	**(*R*)-19a**
1	CH_3CN	Cs_2CO_3 (1)	1.1	150[b]	0.5	5	14	33
2	THF	NaH (2)	1.1	rt	1	14	5	3
3	THF/DMF (9:1)	NaH (2)	1.1	rt	1	32	1	30
4	THF/DMF (9:1)	NaH (2)	1.1	-20	1	11	0	5
5	THF/DMF (9:1)	NaH (2)	1.1	0	1	48	2	25
6[c]	THF/DMF (9:1)	NaH (3)	1.5	0	3	58	2	20

[a] Determined by HPLC-MS analysis [Sunfire C_{18} (4.6×50 mm, 3.5 μm)]. [b]MW heating. [c]Addition of 0.5 eq. of BnBr and 1 eq. of NaH each hour.

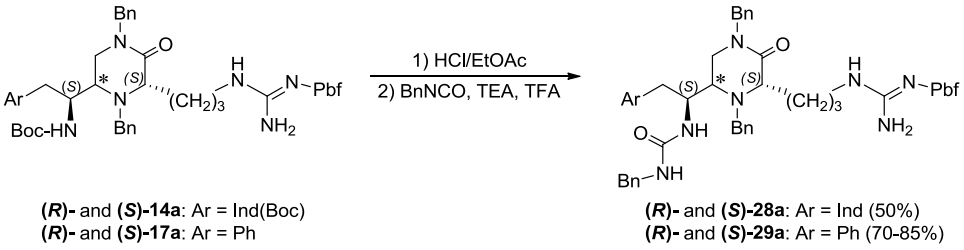
The assignment of the benzylation position was based on $^{1}H,^{1}H$ COSY and $^{1}H,^{13}C$ HSQC and HMBC correlation spectra. The benzyl methylenic protons at N_1 in the piperazine ring and at the guanidino group appeared overlapped in ^{1}H NMR and their assignment was carried out based on their HMBC correlation with the lactamic carbon C_2, at 170-172 ppm, and with the guanidine carbon at 154-156 ppm, respectively. Furthermore, these methylenic protons at the N_1 position appeared as an AB system, with a geminal coupling constant of 14-15 Hz. However, the methylenic protons at the guanidine group appeared as a doublet or doublet of doublets coupled to a NH, with a coupling constant of 5-6 Hz, which moved varying the spectra temperature, and disappeared after stirring with D_2O. These data helped us to assign the structure of the trisubstituted guanidine group in **16a**, **19a**, **22a** and **25a**, with the Pbf protecting group attached to the C=N bond, as described for sulfaguanidines [52]. The HPLC-MS chromatograms of these benzylguanidylated compounds showed two overlapped peaks with the same mass in a (2:1) ratio, which could not be resolved. In spite of the signal overlapping and problems of resolution due to dynamic exchange, two isomers in the same ratio were also observed in the ^{1}H NMR spectra of these compounds in $(CD_3)_2CO$, which were attributed to the interconverting *E*- and *Z*-isomers at the guanidino group [53, 54]. This interconversion could explain the low resolution of the ^{1}HNMR spectra.

The application of the benzylation conditions of entry 6 in Table 2 to the lysine derived N_1-unsubstituted-2-oxopiperazines **(*R*)-** and **(*S*)-**(**10b** and **12b**) led to the corresponding N_1-benzyl derivatives **(*R*)-** and **(*S*)-**(**26b** and **27b**) (Scheme 5), which were isolated in 70-80% yields, without observing benzylation at the lysine protected side chain.



Scheme 5. N_1-Benzylation of the lysine derived 4-substituted-2-oxopiperazines **10b** and **12b**.

In the arginine derived 1,4-dibenzyl-2-oxopiperazines **(*R*)-** and **(*S*)-**(**14a** and **17a**), where the selective removal of the Boc protections without removal of the protection at the basic amino acid side chain was possible, the Boc-HN group of the exocyclic moiety at C_5 was replaced by a benzyl-ureido group. Thus, as shown in Scheme 6, removal of the Boc groups, by treatment with 3N solution of HCl in EtOAc, followed by reaction with benzyl isocyanate in the presence of TEA, gave the corresponding ureas **(*R*)-** and **(*S*)-**(**28a** and **29a**) in 50-85% yield. In all cases, the Arg protection Pbf was not affected by the reactions.

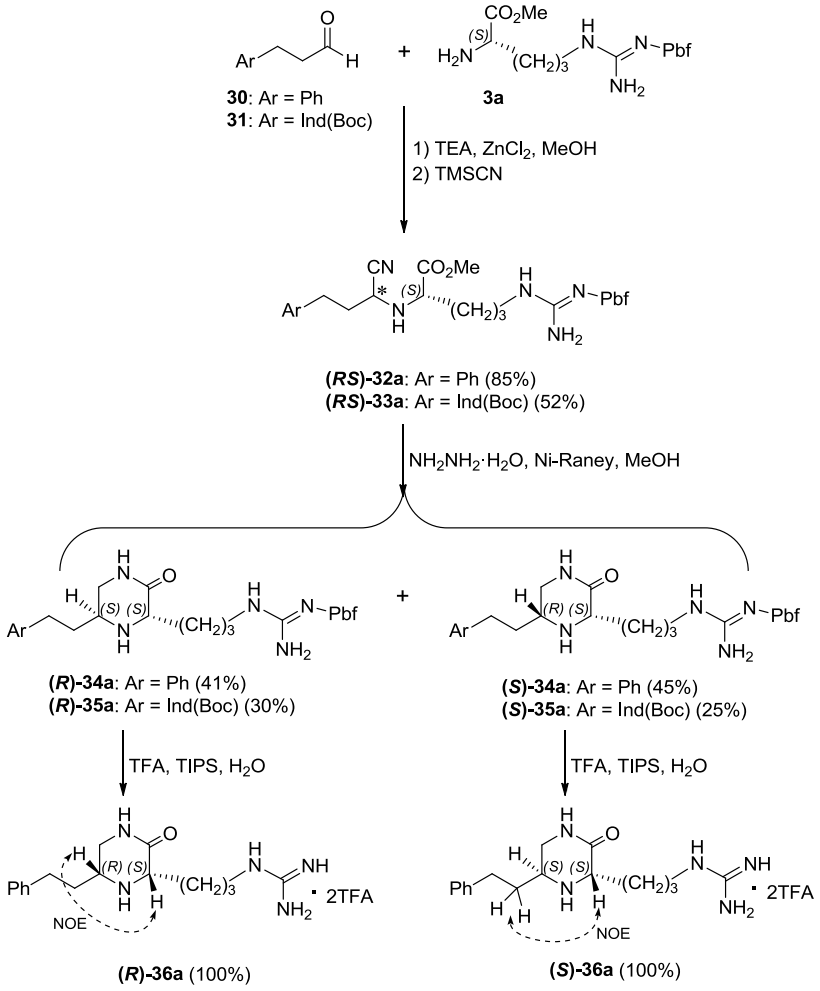


Scheme 6. Synthesis of 2-oxopiperazine derived benzyl-ureas.

It is interesting to note that in the ^{1}H NMR spectra of the (*S*)-epimer of the N_1-benzyl-N_4-*tert*-butoxycarbonylmethyl-2-oxopiperazine derivatives **21a**, **22a**, **24a**, **25a** and **27b**, as well as of the urea derivatives **28a** and **29a**, the 6-H protons appeared as isochronous, indicative, as above commented, of high conformational flexibility in the piperazine ring.

With the aim of studying the influence of the substituent R^4 at position 5 of the 2-oxipiperazines upon the biological activity, aralkyl analogues (R^4 = H) were also synthesized following a similar synthetic strategy to that commented for the tryptophan and phenylalanine derivatives. As in the biological screening of these derivatives, compounds containing the arginine side chain had given better results than those containing the lysine side chain, the new analogues were focused only on those containing arginine. Thus, as shown in Scheme 7, the modified Strecker reaction of aldehydes **30** and **31** with H-Arg(Pbf)-OMe (**3a**) gave the corresponding epimeric mixtures of α-amino nitriles **(*RS*)-32a** and **-33a** in a (1:1) ratio, which could not be separated. The Ni Raney catalyzed hydrogenation of these α-amino nitriles led to the respective 2-oxopiperazine derivatives **(*R*)-** and **(*S*)-34a** and **-35a**, but in yields lower than 40%, due to retro-Strecker and cyano removal side reactions. After studying other catalysts

and hydrogenation conditions, it was deduced that the best reduction conditions were hydrogen transfer from hydrazine monohydrate using Ni Raney as catalyst under refluxing MeOH for 10 min [47]. In this way, the epimeric mixtures **34a** and **35a** were obtained in 55-86% yield and were chromatographically resolved into the (*R*)- and (*S*)-epimers.

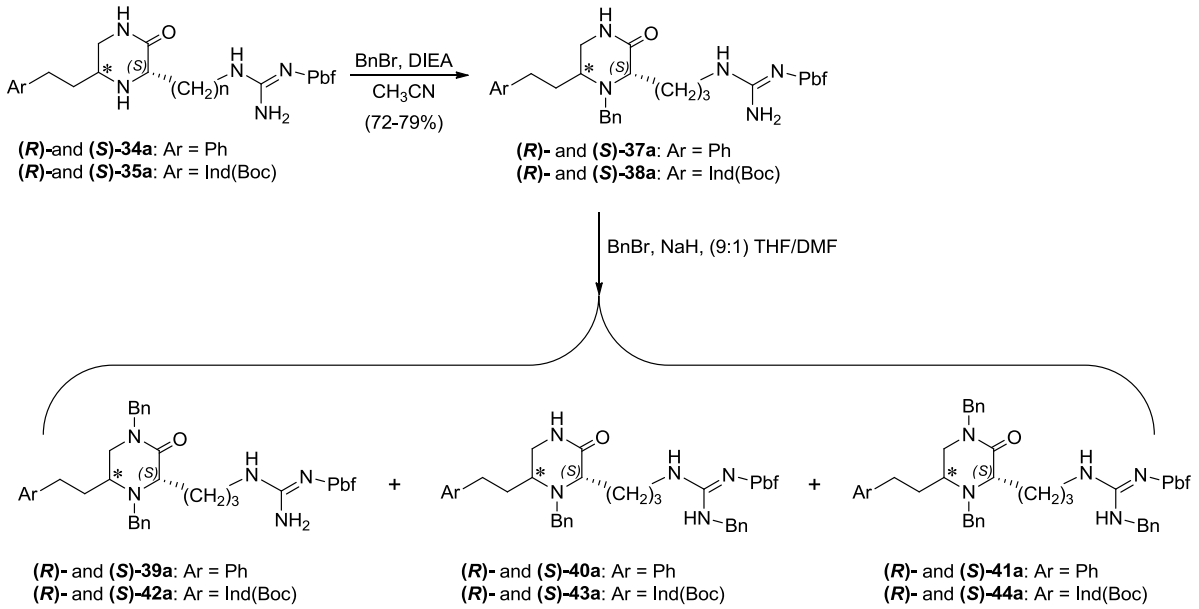


Scheme 7. Synthesis and configuration assignment of the 2-oxopiperazine derivatives **34a** and **35a**.

NOE effects between the 3-H and 5-H protons were not observed in the NOESY 1D spectra of both epimers of the 2-oxopiperazines **34a** and **35a** in different solvents [$CDCl_3$ or $(CD_3)_2CO$] to be used for assignment of configuration at C_5. However, after the removal of the Arg side chain protection Pbf in the 5-phenethyl derivatives **34a**, by treatment with TFA in the presence of triisopropylsilane (TIPS), the NOE effects observed in the NOESY 1D spectra of **(*R*)-** and **(*S*)-36a** in D_2O [Scheme 7, NOE between 3-H and 5-H in **(*R*)-36a** and

between 3-H and 5-CH_2 in **(*S*)-36a**] allowed the indirect assignment of configuration for both epimers in **36a** and in **34a**. In the comparison of the HPLC-MS and NMR data for the pair of epimers **34a**, it was observed that the (*S*)-epimer showed higher t_R than the (*R*) and that the 3-H proton, as well as C_3 and C_5, appeared at a lower field in the (*S*)-epimer than in the (*R*). These differences between the pair of epimers were used for the tentative assignment of the analogue 2-oxopiperazines containing the *N*-Boc-indol-3-yl moiety **35a**.

The application of the reaction conditions previously set up for the regioselective alkylation of the 2-oxopiperazines **7a** to **(*R*)-** and **(*S*)-**(**34a** and **35a**) allowed first the preparation of the 4-benzyl-2-oxopiperazine derivatives **(*R*)-** and **(*S*)-**(**37a** and **38a**) (Scheme 8), and later that of the 1,4-dibenzyl-2-oxopiperazines **(*R*)-** and **(*S*)-**(**39a** and **42a**) (40-50%), along with the tribenzylated derivatives **(*R*)-** and **(*S*)-**(**41a** and **44a**), as minor products (8-14%). As above commented, these tribenzylated derivatives also appeared as two inseparable mixture of E-and Z-benzylguanidyl derivatives in HPLC-MS and NMR. As in the case of the 2-oxopiperazines **15a**, **18a**, **21a** and **24a**, the compounds resulting from benzylation at the N_4-position in the piperazine ring and at the guanidine group **40a** and **43a**, although were detected in the HPLC-MS spectra analyses, they could not be isolated and completely characterized.



Scheme 8. Regioselective benzylations of the 2-oxopiperazines **34a** and **35a**.

2.2. Biological evaluation

2.2.1. PAR1 antagonist activity

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). None of the lysine derived 2-oxopiperazines **6b**, **10b**, **12b**, **26b** and **27b** inhibited the platelet aggregation in this assay. However, as shown in Fig. 2, most of the arginine derived 1-benzyl-4-substituted-2-oxopiperazines showed significant % of inhibition. The low range of variability in the inhibition percentages did not allow to establish clear structure-activity relationships. Thus, the type of aromatic moiety did not significantly affect the inhibition, except for the derivatives containing a benzyl-urea at the exocyclic substituent R^4, wherein the Phe derivatives **29a** were better than the respective Trp derivatives **28a**. In fact, the (*S*)-epimer **(*S*)-29a**, with a 57% of inhibition was the best of the series. Neither the substituent at N_4 (R^2) nor the stereochemistry at C_5 showed a clear influence upon the activity. In the case of stereochemistry, its low influence on the activity could be related to the already commented high flexibility of the 2-oxopiperazine ring, observed in the NMR spectra of most compounds. The comparative analysis of the platelet aggregation inhibition results of this series of 2-oxopiperazine derivatives of general formula **B** with those of our previously reported series of urea analogues **A** [47], shows that the conformational restriction of the 2-oxopiperazine ring does not affect the binding of the compounds to PAR1, as the best compounds of both series displayed similar activity values.

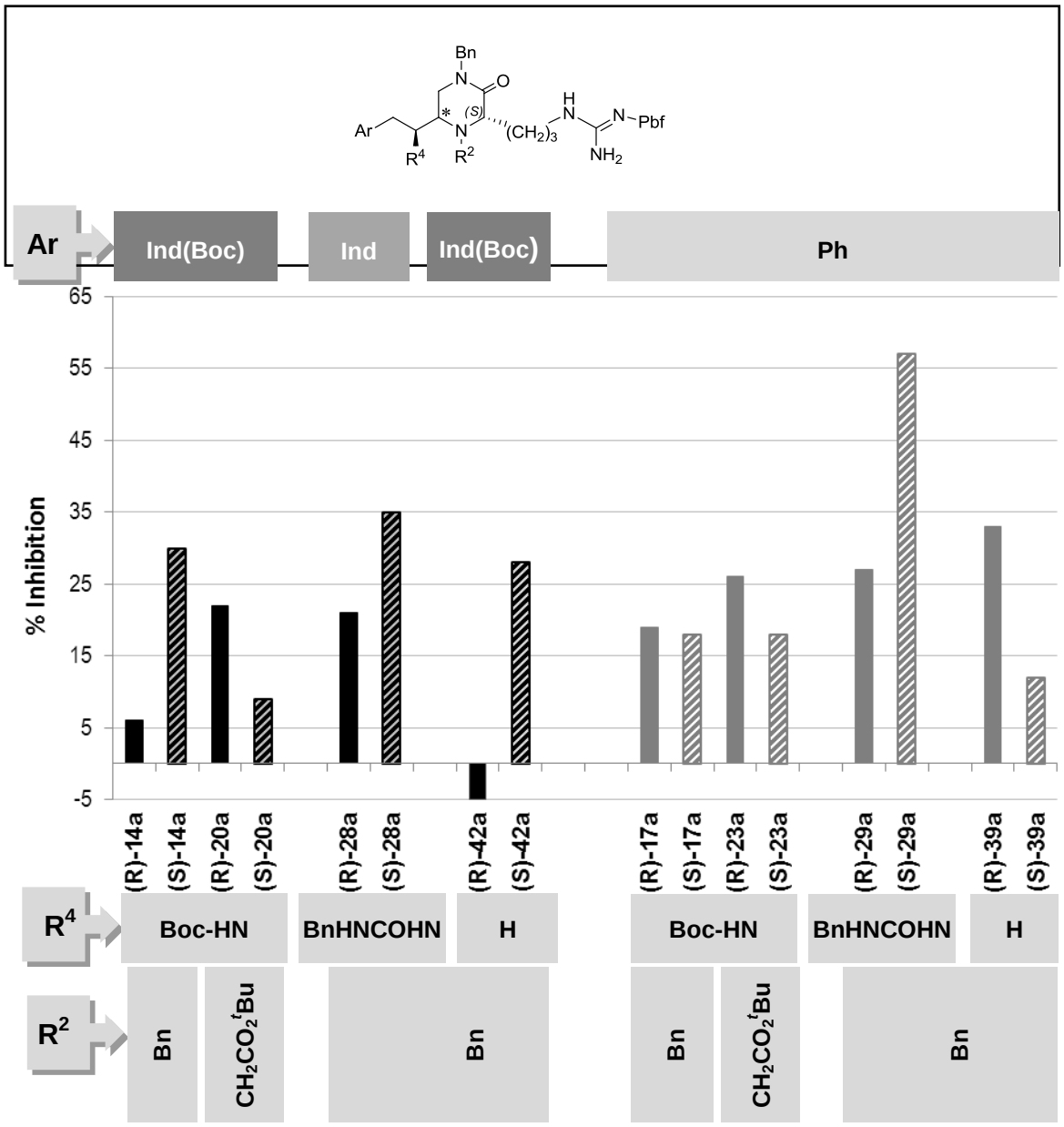


Fig. 2. Inhibition (%) of human platelet aggregation induced by a 30 μM concentration of SFLLRN.

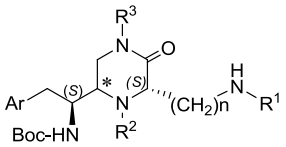
2.2.2. Antitumor activity

All new compounds herein described were evaluated as antitumor agents in a HTS programme, which included evaluation of cytotoxicity on three representative human cancer cell lines: breast (MDA-MB-231), lung (A549), and colon (HT-29), using doxorubicin as

positive control and according to the National Cancer Institute (NCI) protocols. The three cell growth parameters: GI_{50} (concentration that produces 50% growth inhibition), TGI (concentration that produces total growth inhibition), and LC_{50} (concentration that produces 50% of cellular death) were determined from the data analysis, automatically generated by the HTS laboratory information management system. The GI_{50} results of the compounds that displayed values lower than the highest 100 μM concentration are shown in Table 3.

Table 3

GI_{50} values of the cytotoxicity screening



Compound	Ar	Conf. (*)	R^1	n	R^2	R^3	GI_{50} (μM) Breast MDAMB-231	Lung A549	Colon HT-29
(*R*)-10a	Ind(Boc)	(*R*)	$C(NH_2)$=N-Pbf	3	Bn	H	4.44	4.89	3.78
(*S*)-10a	Ind(Boc)	(*S*)	$C(NH_2)$=N-Pbf	3	Bn	H	4.55	5.55	3.78
(*R*)-11a	Ph	(*R*)	$C(NH_2)$=N-Pbf	3	Bn	H	6.83	1.24	5.26
(*S*)-11a	Ph	(*S*)	$C(NH_2)$=N-Pbf	3	Bn	H	6.04	5.39	3.29
(*R*)-12a	Ind(Boc)	(*R*)	$C(NH_2)$=N-Pbf	3	$CH_2CO_2{}^tBu$	H	4.54	3.25	4.98
(*S*)-12a	Ind(Boc)	(*S*)	$C(NH_2)$=N-Pbf	3	$CH_2CO_2{}^tBu$	H	4.87	7.68	2.92
(*R*)-6b	Ind(Boc)	(*R*)	Boc	4	H	H	5.56	>10	5.43
(*S*)-6b	Ind(Boc)	(*S*)	Boc	4	H	H	5.31	9.75	5.43
(*R*)-10b	Ind(Boc)	(*R*)	Boc	4	Bn	H	2.92	4.86	4.58
(*S*)-10b	Ind(Boc)	(*S*)	Boc	4	Bn	H	4.58	5.56	4.45
(*R*)-12b	Ind(Boc)	(*R*)	Boc	4	$CH_2CO_2{}^tBu$	H	2.29	4.57	3.09
(*S*)-12b	Ind(Boc)	(*S*)	Boc	4	$CH_2CO_2{}^tBu$	H	6.72	8.60	5.78
(*R*)-26b	Ind(Boc)	(*R*)	Boc	4	Bn	Bn	3.33	>10	>10
(*S*)-26b	Ind(Boc)	(*S*)	Boc	4	Bn	Bn	1.48	>10	3.83
(*R*)-27b	Ind(Boc)	(*R*)	Boc	4	$CH_2CO_2{}^tBu$	Bn	1.56	>10	4.44
Doxorubicin[a]							0.09	0.07	0.10

[a]Reference drug

As shown in Table 3, the N_1-unsubstituted arginine derivatives (R^3 =H) **10a**-**12a**, as well as most of the tryptophan-lysine pseudodipeptide derived 2-oxopiperazines, displayed μM cytotoxicity in the three cell lines. None of the compounds without the Boc-NH group at the aralkyl moiety at C_5 showed cytotoxicity at the highest assayed concentration of 100 μM. Among the cytotoxic compounds, it is noteworthy to comment the selectivity of the lysine derived 1-benzyl-2-oxopiperazines **(*R*)-**, **(*S*)-26b** and **(*R*)-27b** against breast cancer cells MDAMB-231, for which they were the best compounds of the series. There was not any coincidence between the cytotoxic compounds with those active in the platelet aggregation assay. The lack of correlation between both two types of activities in this series of compounds seems to indicate independent mechanisms of action.

3. Conclusions

A series of pseudodipeptide based chiral 1,3,4,5-tetrasubstituted-2-oxopiperazines has been designed and synthesized as potential PAR1 antagonists by applying a DOS strategy. These highly functionalized piperazine derivatives are prepared through a versatile scheme in four steps from aromatic and basic amino acid derived Ψ[CH(CN)NH]pseudodipeptides. This route involves reduction of the cyano group to build the 2-oxo-piperazine ring, followed by selective functionalization at the N_4-, N_1-positions, and at the exocyclic moiety at position C_5. The selective functionalization at N_4- and N_1-positions, via alkylation, has required the fine tuning of the reaction conditions, particularly those of alkylation at N_1-position in the arginine derivatives, in order to minimize the simultaneous alkylation at the protected arginine guanidino group. In spite of the wide screening of reaction conditions, it has not been possible to completely avoid this side reaction.

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. In this screening, most of the arginine derived 1-benzyl-4-substituted-2-oxopiperazines showed moderate antiaggregant activity. The low range of variability in the platelet inhibition percentages does not allow to establish clear structure-activity relationships. Besides, taking into account our interest in PAR1 antagonists in the field of antitumor agents, all compounds have also been included in a HTS programme for that activity. In this screening some of the N_1-unsubstituted arginine derivatives and most of the tryptophan-lysine pseudodipeptide

derived 2-oxopiperazines, displayed μM cytotoxicity in breast, lung, and colon human cancer cell lines. There is no correlation between the results of PAR1 antagonism and cytotoxicity of the compounds. Therefore, in this series of compounds the mechanisms responsible of both types of activity seems to be independent.

4. **Experimental**

4.1. General

All reagents were of commercial quality. Solvents were dried and purified by standard methods. Analytical TLC was performed on aluminum 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 gradients 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. Gradients of CH_3CN with 0.08% of formic acid (solvent A) in 0.1% of formic acid in H_2O (solvent B) were 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. NMR spectra assignment was based on COSY, HSQC, and HMBC spectra. NOESY 1D spectra were carried out at 400 MHz, using a gradient duration of 0.0005 s and mixing times of 0.5 s and 0.70 s. MW experiments were performed in a MW reactor Emrys^{TM} Synthesizer (Biotage AB).

4.2. General procedure for the synthesis of the Boc-Xaa Ψ[CH(CN)NH]Yaa-OMe pseudodipeptides ***(*****RS*****)-4a,b*** *and* ***-5a***

TEA (1.1 mL, 7.96 mmol) was added to a solution of H-Lys(Boc)-OMe·HCl or H-Arg(Pbf)-OMe·HCl (7.96 mmol) in MeOH (40 mL). After 15 min of stirring at room temperature, the mixture was cooled to -20 ºC and $ZnCl_2$ (542 mg, 3.98 mmol) was added, followed by the addition of the corresponding aldehyde Boc-Xaa-H (Xaa = Phe [55] or Trp(Boc) [56]) (992 mg, 3.98 mmol). The solution was stirred for 1 h at -20 ºC, and the

reaction was heated to 0 ºC. Then, TMSCN was added (896 μL, 7.16 mmol) and the mixture was stirred at 0ºC during 24 h. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc (50 mL). The solution was washed with H_2O (2×25 mL) and brine (25 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using EtOAc in hexane gradient as mobile phase to obtain the epimeric mixtures of pseudodipeptides **(*RS*)-4a**, **(*RS*)-4b** and **(*RS*)-5a**.

*4.2.1. Boc-Trp(Boc)Ψ[CH(CN)NH]Arg(Pbf)-OMe [(***RS***)-4a]*

Foam [4.80 g, 72%, (*R*:*S*) = (1:2)]; HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 60-100% gradient of solvent A in B, 30 min] t_R 14.24 min; ^{1}H NMR [400 MHz, $(CD_3)_2CO$]. **(*R*)-4a** δ (ppm): 1.34 (s, 9H, Boc), 1.43 [s, 6H, $2CH_3$ (Pbf)], 1.67 [s, 9H, Boc (Ind)], 1.68 [m, 2H, γ-H (Arg)], 1.79 [m, 2H, β-H (Arg)], 2.04 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.58 [s, 3H, CH_3 (Pbf)], 2.84 (m, 1H, NH), 2.97 [s, 2H, CH_2 (Pbf)], 3.04 (m, 1H, 3-H), 3.24 [m, 2H, δ-H (Arg)], 3.28 (m, 1H, 3-H), 3.58 [m, 1H, α-H (Arg)], 3.68 (s, 3H, OMe), 4.01 (m, 1H, 1-H), 4.18 (m, 1H, 2-H), 6.34 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 6.47 [m, 3H, $NHC(NH_2)$=N], 7.25 (d, 1H, *J* = 7.5 Hz, Ind), 7.32 (d, 1H, *J* = 7.5 Hz, Ind), 7.58 (m, 1H, Ind), 7.62 (d, 1H, *J* = 7.5 Hz, Ind), 8.13 (d, 1H, *J* = 7.5 Hz, Ind). **(*S*)-4a** δ (ppm): 1.34 (s, 9H, Boc), 1.43 [s, 6H, $2CH_3$ (Pbf)], 1.67 [s, 9H, Boc (Ind)], 1.68 [m, 2H, γ-H (Arg)], 1.79 [m, 2H, β-H (Arg)], 2.04 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.58 [s, 3H, CH_3 (Pbf)], 2.75 (m, 1H, NH), 2.96 [s, 2H, CH_2 (Pbf)], 3.04 (m, 1H, 3-H), 3.24 [m, 2H, δ-H (Arg)], 3.28 (m, 1H, 3-H), 3.41 [m, 1H, α-H (Arg)], 3.70 (s, 3H, OMe), 3.95 (m, 1H, 1-H), 4.24 (m, 1H, 2-H), 6.44 (m, 1H, *NH*Boc), 6.47 [m, 3H, $NHC(NH_2)$=N], 7.25 (d, 1H, *J* = 7.5 Hz, Ind), 7.32 (d, 1H, *J* = 7.5 Hz, Ind), 7.58 (m, 1H, Ind), 7.62 (d, 1H, *J* = 7.5 Hz, Ind), 8.13 (d, 1H, *J* = 7.5 Hz, Ind); ^{13}C NMR (100 MHz, $(CD_3)_2CO$). **(*R*)-4a** δ (ppm): 12.5, 18.1, 19.5 [$3CH_3$ (Pbf)], 27.0 (C_3), 27.2 [C_γ (Arg)], 28.3 [$3CH_3$ (Boc)], 28.5 [$2CH_3$ (Pbf)], 28.7 [$3CH_3$ (Boc)], 31.0 [C_β (Arg)], 41.4 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 52.1 (Ome), 53.3 (C_2), 54.4 (C_1), 59.7 [C_α (Arg)], 79.4, 84.1 [2C (2Boc)], 86.9 [C (Pbf)], 115.6 [CH (Ind)], 117.5 [C (Pbf)], 117.6 [C (Ind)], 119.5 (CN), 119.9, 123.3, 124.8, 125.1 [4CH (Ind)], 125.3 [C (Pbf)], 131.6 [C (Ind)], 132.8, 135.6 [2C (Pbf)], 136.3 [C (Ind)], 138.7 [C (Pbf)], 150.2, 156.3 [2CO (2Boc)], 157.3 [C ($NHC(NH_2)$=N)], 158.9 [C (Pbf)], 174.3 (CO_2). **(*S*)-4a** δ (ppm): 12.5, 18.1, 19.5 [$3CH_3$ (Pbf)], 26.9 (C_3), 27.0 [C_γ (Arg)], 28.3 [$3CH_3$ (Boc)], 28.5 [$2CH_3$ (Pbf)], 28.7 [$3CH_3$ (Boc)], 31.2 [C_β (Arg)], 41.4 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 52.0 (Ome), 53.8 (C_2), 54.0 (C_1), 61.2 [C_α (Arg)], 79.4, 84.1 [2C (2Boc)], 86.9

[C (Pbf)], 115.6 [CH (Ind)], 117.5 [C (Pbf)], 117.7 [C (Ind)], 119.6 (CN), 119.9, 123.3, 124.8, 125.1 [4CH (Ind)], 125.3 [C (Pbf)], 131.4 [C (Ind)], 132.8, 135.6 [2C (Pbf)], 136.3 [C (Ind)], 138.7 [C (Pbf)], 150.2, 156.5 [2CO (2Boc)], 157.3 [C (NHC(NH_2)=N)], 158.9 [C (Pbf)], 174.8 (CO_2); ES-MS *m/z* 838.7 $[M+1]^+$; Anal. calcd. for $C_{42}H_{59}N_7O_9S$: C, 60.20; H, 7.10; N, 11.70. Found: C, 60.11; H, 6.95; N, 11.94.

*4.2.2. Boc-Trp(Boc)Ψ[CH(CN)NH]Lys(Boc)-OMe [(**RS**)-**4b**]*

Foam [4.60 g, 87%, (*R*:*S*) = (1:2)]; HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 70-100% gradient of solvent A in B, 30 min] t_R 11.00 min [**(*R*)-4b]** and 11.36 min [**(*S*)-4b**]; ^{1}H NMR [400 MHz, $(CD_3)_2CO$]. **(*R*)-4b** δ (ppm): 1.35 (s, 9H, Boc), 1.38 [s, 9H, Boc (Lys)], 1.49 [m, 2H, γ-H (Lys)], 1.52 [m, 2H, δ-H (Lys)], 1.67 [m, 9H, Boc (Ind)], 1.81 [m, 2H, β-H (Lys)], 2.82 (m, 1H, NH), 3.06 (m, 1H, 3-H), 3.08 [m, 2H, ε-H (Lys)], 3.31 (dd, 1H, *J* = 3 and 15 Hz, 3-H), 3.58 [dd, 1H, *J* = 6.5 and 12 Hz, α-H (Lys)], 3.71 (s, 3H, OMe), 4.02 (m, 1H, 1-H), 4.20 (m, 1H, 2-H), 5.94 [m, 1H, *NH*Boc (Lys)], 6.34 (d, 1H, *J* = 9 Hz, *NH*Boc), 7.26 (m, 1H, Ind), 7.32 (m, 1H, Ind), 7.59 (m, 1H, Ind), 7.64 (d, 1H, *J* = 7.5 Hz, Ind), 8.14 (d, 1H, *J* = 7.5 Hz, Ind). **(*S*)-4b** δ (ppm): 1.35 (s, 9H, Boc), 1.38 [s, 9H, Boc (Lys)], 1.49 [m, 2H, γ-H (Lys)], 1.52 [m, 2H, δ-H (Lys)], 1.67 [m, 9H, Boc (Ind)], 1.73 [m, 2H, β-H (Lys)], 2.74 (m, 1H, NH), 3.01 (m, 1H, 3-H), 3.08 [m, 2H, ε-H (Lys)], 3.24 (dd, 1H, *J* = 4.5 and 15 Hz, 3-H), 3.40 [dd, 1H, *J* = 8 and 15.5 Hz, α-H (Lys)], 3.72 (s, 3H, OMe), 3.98 (m, 1H, 1-H), 4.25 (m, 1H, 2-H), 5.94 [m, 1H, *NH*Boc (Lys)], 6.45 (d, 1H, *J* = 8.5 Hz, *NH*Boc), 7.26 (m, 1H, Ind), 7.32 (m, 1H, Ind), 7.59 (m, 1H, Ind), 7.69 (d, 1H, *J* = 7.5 Hz, Ind), 8.14 (d, 1H, *J* = 7.5 Hz, Ind); ^{13}C NMR (100 MHz, $(CD_3)_2CO$). **(*R*)-4b** δ (ppm): 23.6 [C_γ (Lys)], 27.3 (C_3), 28.3, 28.7 [9CH_3 (3Boc)], 30.6 [C_δ (Lys)], 33.5 [C_β (Lys)], 40.8 [C_ε (Lys)], 52.1 (OMe), 53.3 (C_2), 54.5 (C_1), 59.9 [C_α (Lys)], 78.3, 79.3, 84.1 [3C (3Boc)], 115.9 [CH (Ind)], 117.6 [C (Ind)], 119.6 (CN), 119.9, 123.3, 124.8, 125.1 [4CH (Ind)], 131.7, 136.4 [2C (Ind)], 150.3, 156.5 [3CO (3Boc)], 174.5 [CO_2]. **(*S*)-4b** δ (ppm): 23.6 [C_γ (Lys)], 27.0 (C_3), 28.3, 28.5 [9CH_3 (3Boc)], 30.6 [C_δ (Lys)], 33.7 [C_β (Lys)], 40.9 [C_ε (Lys)], 52.0 [OMe], 53.7 (C_2), 54.0 (C_1), 61.5 [C_α (Lys)], 78.3, 79.3, 84.1 [3C (3Boc)], 115.9 [CH (Ind)], 117.7 [C (Ind)], 119.7 (CN), 119.9, 123.4, 124.8, 125.1 [4CH (Ind)], 131.5, 136.3 [2C (Ind)], 150.3, 156.5 [3CO (3Boc)], 175.0 (CO_2); ES-MS *m/z* 658.8 $[M+1]^+$; Anal. calcd. for $C_{34}H_{51}N_5O_8$: C, 62.08; H, 7.81; N, 10.65. Found: C, 62.29; H, 7.67; N, 10.78.

*4.2.3. Boc-PheΨ[CH(CN)NH]Arg(Pbf)-OMe [(**RS**)-**5a**]*

Foam [5.30 g, 95%, (*R*:*S)* = (1:1)]; HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 60-100% gradient of solvent A in 30 min] t_R 10.05 min [**(*R*)-5a]** and 10.25 min [**(*S*)-5a**]; ^{1}H NMR [400 MHz, $(CD_3)_2CO$]. **(*R*)-5a** δ (ppm): 1.32 (s, 9H, Boc), 1.44 [s, 6H, $2CH_3$ (Pbf)], 1.66 [m, 2H, γ-H (Arg)], 1.78 [m, 2H, β-H (Arg)], 2.05 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.59 [s, 3H, CH_3 (Pbf)], 2.63 (m, 1H, 3-H), 2.73 (m, 1H, 3-H), 2.85 (m, 1H, NH), 2.98 [s, 2H, CH_2 (Pbf)], 3.24 [m, 2H, δ-H (Arg)], 3.53 [m, 1H, α-H (Arg)], 3.66 (s, 3H, OMe), 3.90 (t, 1H, *J* = 7 Hz, 1-H), 4.08 (m, 1H, 2-H), 6.29 (d, 1H, *J* = 7.5 Hz, *NH*Boc), 6.49 [m, 3H, $NHC(NH_2)=N$], 7.21 (m, 1H, Ph), 7.24-7.32 (m, 4H, Ph). **(*S*)-5a** δ (ppm): 1.32 (s, 9H, Boc), 1.44 [s, 6H, $2CH_3$ (Pbf)], 1.66 [m, 2H, γ-H (Arg)], 1.78 [m, 2H, β-H (Arg)], 2.05 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.59 [s, 3H, CH_3 (Pbf)], 2.63 (m, 1H, 3-H), 2.73 (m, 1H, 3-H), 2.81 (m, 1H, NH), 2.98 [s, 2H, CH_2 (Pbf)], 3.24 [m, 2H, δ-H (Arg)], 3.36 [m, 1H, α-H (Arg)], 3.69 (s, 3H, OMe), 3.84 (dd, 1H, *J* = 4.5 and 11 Hz, 1-H), 4.08 (m, 1H, 2-H), 6.38 (d, 1H, *J* = 9 Hz, *NH*Boc), 6.49 [m, 3H, $NHC(NH_2)=N$], 7.21 (m, 1H, Ph), 7.24-7.32 (m, 4H, Ph); ^{13}C NMR (100 MHz, $(CD_3)_2CO$). **(*R*)-5a** δ (ppm): 12.5, 18.1, 19.4 [$3CH_3$ (Pbf)], 26.6 [C_γ (Arg)], 28.5 [$2CH_3$ (Pbf)], 28.7 [$3CH_3$ (Boc)], 31.0 [C_β (Arg)], 37.3 (C_3), 41.3 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 52.1 (Ome), 53.8 (C_2), 54.7 (C_1), 59.5 [C_α (Arg)], 79.3 [C (Boc)], 86.6, 117.5 [2C (Pbf)], 119.3 (CN), 125.3 [C (Pbf)], 127.1, 129.1, 130.8 [5CH (Ph)], 132.8, 135.5, 138.7 [3C (Pbf)], 139.4 [C (Ph)], 156.3 [CO (Boc)], 157.9 [C ($NHC(NH_2)=N$)], 158.9 [C (Pbf)], 174.4 [CO_2]. **(*S*)-5a** δ (ppm): 12.5, 18.1, 19.4 [$3CH_3$ (Pbf)], 26.0 [C_γ (Arg)], 28.5 [$2CH_3$ (Pbf)], 28.7 [$3CH_3$ (Boc)], 31.1 [C_β (Arg)], 37.1 (C_3), 41.3 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 52.0 (Ome), 54.5 (C_2), 54.5 (C_1), 60.9 [C_α (Arg)], 79.3 [C (Boc)], 86.6, 117.5 [2C (Pbf)], 119.4 (CN), 125.3 [C (Pbf)], 127.2, 129.2, 130.8 [5CH (Ph)], 132.8, 135.5, 138.7 [3C (Pbf)], 139.4 [C (Ph)], 156.3 [CO (Boc)], 157.9 [C ($NHC(NH_2)=N$)], 158.9 [C (Pbf)], 174.7 (CO_2).; ES-MS *m/z* 699.6 $[M+1]^+$; Anal. calcd. for $C_{35}H_{50}N_6O_7S$: C, 60.15; H, 7.21; N, 12.03. Found: C, 60.21; H, 7.39; N, 11.91.

4.3. General procedure for the synthesis of the 2-oxopiperazine derivatives **(R)-** *and* **(S)-6a,b** *and* **-7a**

Raney-Ni (1.50 g) was added to a solution of the corresponding epimeric mixture of pseudodipeptides **(*RS*)-4a,b** and **-5a** (2.88 mmol) in MeOH (15 mL) and the mixture was hydrogenated at 1 atm of H_2 at room temperature for 12 h. Afterward, the reaction mixture was filtered over celite and the solvent was evaporated under reduced pressure. The residue

was purified by flash chromatography, by using MeOH in CH_2Cl_2 gradient as mobile phase to obtain the desired resolved 2-oxopiperazines **(*R*)-** and **(*S*)-6a,b** and **-7a**.

*4.3.1. (5*R,*3*S*)-5-((*S*)-1-((*tert-*Butoxycarbonyl)amino-2-(1-(*tert-*butoxycarbonyl)-indol-3-yl) ethyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)-propyl)-2-oxopiperazine [**(R)-6a**][1]*

White solid (933 mg, 40%); $[\alpha]_D^{20}$ -5.1 (*c* 1.0, CH_2Cl_2); Mp: 118-120 ºC (EtOAc/hexane); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), isocratic 50% of solvent A] t_R 8.70 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.30 (s, 9H, Boc), 1.42 [s, 6H, $2CH_3$ (Pbf)], 1.66 [s, 3H, Boc (Ind)], 1.69 [m, 1H, γ-H (Arg)], 1.73 [m, 1H, γ-H (Arg)], 1.82 [m, 1H, β-H (Arg)], 1.89 [m, 1H, β-H (Arg)], 2.04 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.59 [s, 3H, CH_3 (Pbf)], 2.87 (m, 1H, *CH_2*-Ind), 2.95 [s, 2H, CH_2 (Pbf)], 3.19 (m, 1H, 5-H), 3.27 (m, 1H, 6-H), 3.28 [m, 2H, δ-H (Arg)], 3.35 [m, 1H, 3-H], 3.40 (m, 1H, *CH_2*-Ind), 3.48 (dt, 1H, *J* = 4 and 11.5 Hz, 6-H), 3.90 (m, 1H, 5-CH), 6.06 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 6.58 [m, 3H, $NHC(NH_2)=N$], 6.90 (s, 1H, 1-H), 7.22 (t, 1H, *J* = 7.5 Hz, Ind), 7.30 (t, 1H, *J* = 7.5 Hz, Ind), 7.51 (m, 1H, Ind), 7.61 (d, 1H, *J* = 7.5 Hz, Ind), 8.12 (d, 1H, *J* = 7.5 Hz, Ind). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 12.5, 18.2, 19.5 [$3CH_3$ (Pbf)], 27.7 [C_γ (Arg) and *CH_2*-Ind], 28.3 [$3CH_3$ (Boc)], 28.6 [$2CH_3$ (Pbf)], 28.7 [$3CH_3$ (Boc)], 29.6 [C_β (Arg)], 41.5 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 46.1 (C_6), 51.8 (C_5), 53.6 (C_5-*CH*), 56.7 (C_3), 79.4, 83.9 [2C (2Boc)], 86.9 [C (Pbf)], 115.6 [CH (Ind)], 117.4 [C (Pbf)], 118.9 [C (Ind)], 120.1, 123.2, 124.3, 124.9 [4CH (Ind)], 125.3 [C (Pbf)], 132.0 [C (Ind)], 132.8, 135.7 [2C (Pbf)], 136.4 [C (Ind)], 138.7 [C (Pbf)], 150.3, 156.8 [2CO (2Boc)], 157.3 [C ($NHC(NH_2)=N$)], 158.9 [C (Pbf)], 173.0 (C_2); ES-MS *m/z* 810.9 $[M+1]^+$; Anal. calcd. for $C_{41}H_{59}N_7O_8S$: C, 60.79; H, 7.34; N, 12.10. Found: C, 60.52; H, 7.51; N, 12.27.

*4.3.2. (5*S,*3*S*)-5-((*S*)-1-((*tert-*Butoxycarbonyl)amino-2-(1-(*tert-*butoxycarbonyl)-indol-3-yl) ethyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)-propyl)-2-oxopiperazine [**(S)-6a**]*

White solid (467 mg, 20%); $[\alpha]_D^{20}$ -15 (*c* 1.1, CH_2Cl_2); Mp: 118-120 ºC (EtOAc/hexane); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), isocratic 50% of solvent A] t_R 9.05 min; ^{1}H

[1] As the 2-oxopiperazine ring is the common structure of the compounds, we have preferred to give preference to this ring in the nomenclature of the compounds with respect to other senior groups or skeletons in the IUPAC nomenclature system.

NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.33 (s, 9H, Boc), 1.42 [s, 6H, $2CH_3$ (Pbf)], 1.66 [s, 3H, Boc (Ind)], 1.68 [m, 2H, γ-H (Arg)], 1.70 [m, 1H, β-H (Arg)], 1.92 [m, 1H, β-H (Arg)], 2.05 [s, 3H, CH_3 (Pbf)], 2.51 [s, 3H, CH_3 (Pbf)], 2.59 [s, 3H, CH_3 (Pbf)], 2.96 (m, 1H, *CH_2*-Ind), 2.97 [s, 2H, CH_2 (Pbf)], 3.07 (m, 1H, *CH_2*-Ind), 3.12 (m, 1H, 5-H), 3.24 [m, 2H, δ-H (Arg)], 3.27 (m, 2H, 6-H), 3.32 [m, 1H, 3-H], 4.00 (m, 1H, 5-CH), 6.09 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 6.53 [m, 3H, NHC(NH_2)=N], 6.85 (s, 1H, 1-H), 7.23 (t, 1H, *J* = 7.5 Hz, Ind), 7.31 (t, 1H, *J* = 7.5 Hz, Ind), 7.54 (m, 1H, Ind), 7.68 (d, 1H, *J* = 7.5 Hz, Ind), 8.12 (d, 1H, *J* = 7.5 Hz, Ind). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 12.6, 18.2, 19.5 [$3CH_3$ (Pbf)], 26.5 [C_γ (Arg) and *CH_2*-Ph], 27.9 [*CH_2*-Ind], 28.3 [$3CH_3$ (Boc)], 28.6 [$2CH_3$ (Pbf)], 28.8 [$3CH_3$ (Boc)], 29.6 [C_β (Arg)], 41.6 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 46.1 (C_6), 53.0 (C_5-*CH*), 55.7 (C_5), 59.2 (C_3), 78.9, 84.0 [2C (2Boc)], 86.9 [C (Pbf)], 115.8 [CH (Ind)], 117.4 [C (Pbf)], 118.5 [C (Ind)], 120.1, 123.2, 124.5, 125.0 [4CH (Ind)], 125.4 [C (Pbf)], 131.8 [C (Ind)], 132.8, 135.6 [2C (Pbf)], 136.4 [C (Ind)], 138.7 [C (Pbf)], 150.3, 156.8 [2CO (2Boc)], 157.3 [C (NHC(NH_2)=N)], 158.9 [C (Pbf)], 171.8 (C_2]; ES-MS *m/z* 810.9 $[M+1]^+$; Anal. calcd. for $C_{41}H_{59}N_7O_8S$: C, 60.79; H, 7.34; N, 12.10. Found: C, 60.97; H, 7.15; N, 12.32.

*4.3.3. (5*R,*3*S*)-5-((*S*)-1-((*tert-*Butoxycarbonyl)amino-2-(1-(*tert-*butoxycarbonyl)-indol-3-yl)-ethyl-3-(4-((*tert-*butoxycarbonyl)amino)butyl)-2-oxopiperazine [(**R**)-6b]*

White solid (726 mg, 40%); $[\alpha]_D^{20}$ -12 (*c* 1.6, CH_2Cl_2); Mp: 84-86 ºC (EtOAc/hexane); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 30-100% gradient of solvent A in B, 30 min] t_R 13.89 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.31 (s, 9H, Boc), 1.37 [s, 9H, Boc (Lys)], 1.52 [m, 2H, γ-H (Lys)], 1.54 [m, 2H, δ-H (Lys)], 1.66 [s, 9H, Boc (Ind)], 1.69 [m, 1H, β-H (Lys)], 1.84 [m, 1H, β-H (Lys)], 2.40 (m, 1H, 4-H), 2.89 (m, 1H, *CH_2*-Ind), 3.10 [m, 2H, ε-H (Lys)], 3.17 (m, 1H, 5-H), 3.26 (m, 1H, 6-H), 3.31 (m, 1H, 3-H), 3.43 (m, 1H, *CH_2*-Ind), 3.47 (m, 1H, 6-H), 3.87 (ddd, 1H, *J* = 3, 9.5 and 18.5 Hz, 5-CH), 5.95 [m, 1H, *NH*Boc (Lys)], 6.01 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 6.78 (s, 1H, 1-H), 7.23 (t, 1H, *J* = 7.5 Hz, Ind), 7.30 (t, 1H, *J* = 7.5 Hz, Ind), 7.51 (m, 1H, Ind), 7.63 (d, 1H, *J* = 7.5 Hz, Ind), 8.12 (d, 1H, *J* = 7.5 Hz, Ind). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 24.6 [C_γ (Lys)], 27.7 [*CH_2*-Ind], 28.3, 28.6, 28.7 [$9CH_3$ (3Boc)], 30.7 [C_δ (Lys)], 31.2 [C_β (Lys)], 41.2 [C_ε (Lys)], 46.4 (C_6), 52.0 (C_5), 53.6 (C_5-*CH*), 57.7 (C_3), 78.3, 78.7, 83.9 [3C (3Boc)], 115.8 [CH (Ind)], 118.9 [C (Ind)], 120.1, 123.1, 124.3, 124.9 [4CH (Ind)], 132.1, 136.3 [2C (Ind)], 150.3, 156.6 [3CO (3Boc)], 173.0 (C_2); ES-MS *m/z* 630.8 $[M+1]^+$; Anal. calcd. for $C_{33}H_{51}N_5O_7$: C, 62.93; H, 8.16; N, 11.12. Found: C, 62.66; H, 8.37; N, 10.95.

*4.3.4. (5*S,*3*S*)-5-((*S*)-1-((*tert-*Butoxycarbonyl)amino-2-(1-(*tert-*butoxycarbonyl)-indol-3-yl)-ethyl-3-(4-((*tert-*butoxycarbonyl)amino)butyl)-2-oxopiperazine [**(S)-6b**]*

White solid (363 mg, 20%); $[\alpha]_D^{20}$ -10 (*c* 1.4, CH_2Cl_2); Mp: 76-78 ºC (EtOAc/hexane); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 30-100% gradient of solvent A in 30 min] t_R 13.56 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.35 (s, 9H, Boc), 1.39 [s, 9H, Boc (Lys)], 1.48 [m, 2H, γ-H (Lys)], 1.50 [m, 2H, δ-H (Lys)], 1.59 [m, 1H, β-H (Lys)], 1.67 [s, 9H, Boc (Ind)], 1.94 [m, 1H, β-H (Lys)], 2.99 (dd, 1H, *J* = 8 and 14.5 Hz, *CH*$_2$-Ind), 3.08 [m, 2H, ε-H (Lys)], 3.10 (m, 1H, *CH*$_2$-Ind), 3.12 (m, 1H, 5-H), 3.26 (m, 2H, 6-H), 3.27 (m, 1H, 3-H), 4.00 (m, 1H, 5-CH), 5.94 [m, 1H, *NH*Boc (Lys)], 6.06 (d, 1H, *J* = 9 Hz, *NH*Boc), 6.73 (s, 1H, 1-H), 7.25 (t, 1H, *J* = 7.5 Hz, Ind), 7.31 (t, 1H, *J* = 7.5 Hz, Ind), 7.55 (m, 1H, Ind), 7.70 (d, 1H, *J* = 7.5 Hz, Ind), 8.13 (d, 1H, *J* = 7.5 Hz, Ind). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 23.9 [C_γ (Lys)], 28.0 [*CH*$_2$-Ind], 28.3, 28.6, 28.8 [9CH_3 (3Boc)], 31.0 [C_δ (Lys)], 32.6 [C_β (Lys)], 41.1 [C_ε (Lys)], 46.2 (C_6), 53.0 (C_5-*CH*), 55.5 (C_5), 59.7 (C_3), 78.3, 78.9, 84.0 [3C (3Boc)], 115.6 [CH (Ind)], 118.5 [C (Ind)], 120.1, 123.3, 124.6, 125.0 [4CH (Ind)], 131.8, 136.4 [2C (Ind)], 150.3, 156.7 [3CO (3Boc)], 171.7 (C_2); ES-MS *m/z* 630.8 $[M+1]^+$; Anal. calcd. for $C_{33}H_{51}N_5O_7$: C, 62.93; H, 8.16; N, 11.12. Found: C, 62.83; H, 8.02; N, 10.90.

*4.3.5. (5*R,*3*S*)-5-((*S*)-1-(*tert-*Butoxycarbonyl)amino-2-phenylethyl)-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino) propyl)-2-oxopiperazine [**(R)-7a**]*

White solid (754 mg, 39%); $[\alpha]_D^{20}$ -6 (*c* 1.0, CH_2Cl_2); Mp: 136-138 ºC (EtOAc/hexane); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 10-100% gradient of solvent A in B, 30 min] t_R 18.05 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.31 (s, 9H, Boc), 1.44 [s, 6H, 2CH_3 (Pbf)], 1.60 [m, 2H, γ-H (Arg)], 1.70 [m, 1H, β-H (Arg)], 1.80 [m, 1H, β-H (Arg)], 2.06 [s, 3H, CH_3 (Pbf)], 2.35 (m, 1H, 4-H), 2.51 [s, 3H, CH_3 (Pbf)], 2.59 [s, 3H, CH_3 (Pbf)], 2.75 (dd, 1H, *J* = 10 and 14 Hz, *CH*$_2$-Ph), 2.98 [s, 2H, CH_2 (Pbf)], 3.07 (m, 1H, 5-H), 3.18 [m, 1H, δ-H (Arg)], 3.23 (m, 1H, *CH*$_2$-Ph), 3.24 (m, 1H, 6-H), 3.30 [m, 2H, δ-H (Arg) and 3-H], 3.43 (dt, 1H, *J* = 4 and 12 Hz, 6-H), 3.83 (ddd, 1H, *J* = 4, 9.5 and 18 Hz, 5-CH), 5.96 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 6.58 [m, 3H, NHC(NH_2)=N], 6.85 (s, 1H, 1-H), 7.16 (m, 1H, Ph), 7.20-7.28 (m, 4H, Ph). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 13.2, 18.9, 20.2 [3CH_3 (Pbf)], 28.0 [C_γ (Arg)], 29.2 [3CH_3 (Boc)], 29.4 [2CH_3 (Pbf)], 29.5 [C_β (Arg)], 38.8 [*CH*$_2$-Ph], 42.2 [C_δ (Arg)], 44.3 [CH_2 (Pbf)], 46.6 (C_6), 52.4 (C_5), 55.8 (C_5-*CH*), 57.4 (C_3), 79.4 [C (Boc)], 87.6,

118.1 [2C (Pbf)], 125.9 [C (Pbf)], 127.4, 129.6, 130.9 [5CH (Ph)], 133.5, 136.6, 139.4 [3C (Pbf)], 140.9 [C (Ph)], 158.0 [CO (Boc)], 157.3 [C (NHC(NH_2)=N)], 159.5 [C (Pbf)], 173.6 [C_2]; ES-MS *m/z* 671.6 $[M+1]^+$; Anal. calcd. for $C_{34}H_{50}N_6O_6S$: C, 60.87; H, 7.51; N, 12.53. Found: C, 60.98; H; 7.73; N, 12.29.

*4.3.6. (5*S,*3*S*)-5-((*S*)-1-(*tert*-Butoxycarbonyl)amino-2-phenylethyl)-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino) propyl)-2-oxopiperazine [(**S**)-7a]*

White solid (792 mg, 41%); $[\alpha]_D^{20}$ -27 (*c* 1.0, CH_2Cl_2); Mp: 138-140 ºC (EtOAc/hexane); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 10-100% gradient of solvent A in B,30 min] t_R 18.05 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.31 (s, 9H, Boc), 1.44 [s, 6H, $2CH_3$ (Pbf)], 1.60 [m, 2H, γ-H (Arg)], 1.80 [m, 1H, β-H (Arg)], 1.90 [m, 1H, β-H (Arg)], 2.06 [s, 3H, CH_3 (Pbf)], 2.51 [s, 3H, CH_3 (Pbf)], 2.59 [s, 3H, CH_3 (Pbf)], 2.82 (m, 1H, *CH_2*-Ph), 2.96 (m, 1H, *CH_2*-Ph), 3.00 [s, 2H, CH_2 (Pbf)], 3.02 (m, 1H, 5-H), 3.22 [m, 2H, δ-H (Arg)], 3.24 (m, 2H, 6-H), 3.28 [m, 1H, 3-H], 3.87 (m, 1H, 5-CH), 5.98 (d, 1H, *J* = 9 Hz, *NH*Boc), 6.52 [m, 3H, NHC(NH_2)=N], 6.82 (s, 1H, 1-H), 7.18 (m, 1H, Ph), 7.23-7.33 (m, 4H, Ph). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 13.2, 18.9, 20.2 [$3CH_3$ (Pbf)], 27.1 [C_γ (Arg)], 29.2 [$2CH_3$ (Pbf)], 29.4 [$3CH_3$ (Boc)], 29.6 [C_β (Arg)], 39.1 [*CH_2*-Ph], 41.2 [C_δ (Arg)], 44.3 [CH_2 (Pbf)], 46.8 (C_6), 55.2 [C_5-*CH*], 56.2 (C_5), 59.9 (C_3), 79.5 [C (Boc)], 87.6, 118.1 [2C (Pbf)], 125.9 [C (Pbf)], 127.6, 129.7, 130.8 [5CH (Ph)], 133.5, 136.6, 139.4 [3C (Pbf)], 140.5 [C (Ph)], 157.3 [CO (Boc)], 158.0 [C (NHC(NH_2)=N)], 159.6 [C (Pbf)], 172.5 (C_2); ES-MS *m/z* 671.2 $[M+1]^+$; Anal. calcd. for $C_{34}H_{50}N_6O_6S$: C, 60.87; H, 7.51; N, 12.53. Found: C, 60.69; H, 7.69; N, 12.68.

*4.4. General procedure for the synthesis of the 3,6-dioxooctahydroimidazo[1,5-*a*]pyrazines (**R**)- and (**S**)-8a and -9a*

The corresponding 2-oxopiperazine **(R)-** and **(S)-6a** and **-7a** (0.31 mmol) was dissolved in 3.4 N solution of HCl in EtOAc (5 mL) and the mixture was stirred at room temperature for 30 min. Afterward, the solvent was evaporated to dryness, the residue was dissolved in CH_3CN/H_2O (1:3, 2 mL) and the solution was lyophilized. Then, TEA (87 μL, 0.62 mmol) was added to a solution of the lyophilized powder in CH_2Cl_2 (10 mL). After 15 min of stirring at 0 ºC, TEA (101 μL, 0.73 mmol) and triphosgene (36 mg, 0.12 mmol) were successively added. The mixture was stirred at 0 ºC for 2 h and the reaction was diluted CH_2Cl_2 (10 mL).

The solution was successively washed with H_2O (2×5 mL) and brine (5 mL), dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography, using MeOH in CH_2Cl_2 gradient as mobile phase to obtain the respective 3,6-dioxooctahydroimidazo[1,5-a]pyrazine **(R)-** and **(S)-8a** and **-9a**.

*4.4.1. (1*S,*5*S,*8a*R*)-1-(Indol-3-yl)methyl-5-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydro-benzofuran-5-yl)sulfonyl)guanidino) propyl)-3,6-dioxooctahydroimidazo[1,5-*a*]pyrazine [**(R)-8a**]*

Foam (118 mg, 60%); $[\alpha]_D^{20}$ -4.4 (*c* 0.8, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 10-60% gradient of solvent A in B, 30 min] t_R 27.20 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.42 [s, 6H, $2CH_3$ (Pbf)], 1.58 [m, 2H, γ-H (Arg)], 1.70 [m, 1H, β-H (Arg)], 1.90 [m, 1H, β-H (Arg)], 2.03 [s, 3H, CH_3 (Pbf)], 2.48 [s, 3H, CH_3 (Pbf)], 2.56 [s, 3H, CH_3 (Pbf)], 2.97 [s, 2H, CH_2 (Pbf)], 3.04 (d, 2H, *J* = 8.5 Hz, 1-CH_2), 3.21 [m, 2H, δ-H (Arg)], 3.30 (dt, 1H, *J* = 4.5 and 11.5 Hz, 8-H), 3.68 (t, 1H, *J* = 11.5 Hz, 8-H), 4.00 (ddd, 1H, *J* = 4.5, 7.5 and 11.5 Hz, 8a-H), 4.14 (dd, 1H, *J* = 4 and 9.5 Hz, 5-H), 4.41 (q, 1H, *J* = 7.5 Hz, 1-H), 5.94 (bs, 1H, 2-H), 6.50 [m, 3H, $NHC(NH_2)=N$], 7.03 (dd, 1H, *J* = 4 and 11 Hz, Ind), 7.10 (m, 2H, 1-H and Ind), 7.31 (d, 1H, *J* = 2.5 Hz, Ind), 7.41 (d, 1H, *J* = 8 Hz, Ind), 7.61 (d, 1H, *J* = 7.5 Hz, Ind), 10.18 [m, 1H, NH (Ind)]; ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 12.5, 18.2, 19.5 [$3CH_3$ (Pbf)], 25.7 [1-CH_2], 26.3 [C_γ (Arg)], 28.7 [$2CH_3$ (Pbf)], 30.5 [C_β (Arg)], 40.6 (C_8), 41.2 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 52.1 (C_{8a}), 53.7 (C_5 and C_1), 86.9 [C (Pbf)], 111.5 [C (Ind)], 112.3 [CH (Ind)], 117.4 [C (Pbf)], 119.2, 119.6, 122.4, 123.8 [4CH (Ind)], 125.3 [C (Pbf)], 128.2 [C (Ind)], 132.8, 135.8 [2C (Pbf)], 137.7 [C (Ind)], 138.7 [C (Pbf)], 157.3 [C ($NHC(NH_2)=N$)], 158.8 [C (Pbf)], 161.8 (C_3), 170.0 (C_6); ES-MS *m/z* 636.6 $[M+1]^+$; Anal. calcd. for $C_{32}H_{41}N_7O_5S$: C, 60.45; H, 6.50; N, 15.42. Found: C, 60.64; H, 6.75; N, 15.20.

*4.4.2. (1*S,*5*S,*8a*S*)-1-(Indol-3-yl)methyl-5-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydro-benzofuran-5-yl)sulfonyl)guanidino) propyl)-3,6-dioxooctahydroimidazo[1,5-*a*]pyrazine [**(S)-8a**]*

Foam (118 mg, 60%); $[\alpha]_D^{20}$ -4.6 (*c* 0.6, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 10-60% gradient of solvent A in B, 30 min] t_R 27.39 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.42 [s, 6H, $2CH_3$ (Pbf)], 1.40 [m, 2H, γ-H (Arg)], 1.65 [m, 1H, β-H (Arg)], 1.96 [m, 1H, β-H (Arg)], 2.04 [s, 3H, CH_3 (Pbf)], 2.42 [ddd, 1H, *J* = 5, 10.5 and 18.5

Hz, δ-H (Arg)], 2.48 [s, 3H, CH_3 (Pbf)], 2.56 [s, 3H, CH_3 (Pbf)], 2.78 (ddd, 1H, *J* = 3.5, 5.5 and 11.5 Hz, 8-H), 2.97 [s, 2H, CH_2 (Pbf)], 3.02 (m, 1H, 1-CH_2), 3.06 (m, 1H, 8-H), 3.16 [m, 1H, δ-H (Arg)], 3.17 (m, 1H, 1-CH_2), 3.52 (dt, 1H, *J* = 3.5 and 10 Hz, 8a-H), 3.85 (m, 1H, 1-H), 3.86 (m, 1H, 5-H), 6.00 (m, 1H, 2-H), 6.27 [m, 1H, NHC(NH_2)=N], 6.50 [m, 2H, NHC(NH_2)=N], 7.02 (ddd, 1H, *J* = 1, 7 and 8 Hz, Ind), 7.03 (m, 1H, 1-H), 7.11 (ddd, 1H, *J* = 1, 7 and 8 Hz, Ind), 7.29 (d, 1H, *J* = 2.5 Hz, Ind), 7.40 (dt, 1H, *J* = 1 and 8 Hz, Ind), 7.61 (dd, 1H, *J* = 1 and 8 Hz, Ind), 10.18 [m, 1H, NH (Ind)]; ^{13}C NMR (100 MHz, $(CD_3)_2CO$) *δ* (ppm): 12.5, 18.2, 19.5 [3CH_3 (Pbf)], 24.8 [C_γ (Arg)], 28.3 [C_β (Arg)], 28.7 [2CH_3 (Pbf)], 30.7 [1-CH_2], 41.4 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 44.9 (C_8), 55.9 (C_{8a}), 57.9 (C_5), 59.7 (C_1), 86.9 [C (Pbf)], 110.9 [C (Ind)], 112.3 [CH (Ind)], 117.4 [C (Pbf)], 119.2, 119.7, 122.3, 124.3 [4CH (Ind)], 125.2 [C (Pbf)], 128.4 [C (Ind)], 132.8, 135.6 [2C (Pbf)], 137.6 [C (Ind)], 138.7 [C (Pbf)], 157.2 [C (NHC(NH_2)=N)], 158.8 [C (Pbf)], 161.0 (C_3), 170.2 (C_6); ES-MS *m/z* 636.6 $[M+1]^+$; Anal. calcd. for $C_{32}H_{41}N_7O_5S$: C, 60.45; H, 6.50; N, 15.42. Found: C, 60.59; H, 6.24; N, 15.63.

*4.4.3. (1*S,*5*S,*8a*R*)-5-(3-(2-((2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-guanidino)propyl)-1-phenylmethyl-3,6-dioxooctahydroimidazo[1,5*-a*]pyrazine [(**R**)-9a]*

Foam (94 mg, 51%); $[\alpha]_D^{20}$ +4.1 (*c* 1.0, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 10-100% gradient of solvent A in 30 min] t_R 20.41 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) *δ* (ppm): 1.44 [s, 6H, 2CH_3 (Pbf)], 1.64 [m, 2H, γ-H (Arg)], 1.90 [m, 2H, β-H (Arg)], 2.04 [s, 3H, CH_3 (Pbf)], 2.49 [s, 3H, CH_3 (Pbf)], 2.57 [s, 3H, CH_3 (Pbf)], 2.87 (m, 2H, 1-CH_2), 2.98 [s, 2H, CH_2 (Pbf)], 3.19 [m, 2H, δ-H (Arg)], 3.27 (ddd, 1H, *J* = 4, 5 and 11.5 Hz, 8-H), 3.65 (t, 1H, *J* = 11.5 Hz, 8-H), 3.97 (ddd, 1H, *J* = 4, 7 and 11.5 Hz, 8a-H), 4.13 (dd, 1H, *J* = 4 and 9.5 Hz, 5-H), 4.29 (q, 1H, *J* = 7 Hz, 1-H), 5.86 (m, 1H, 2-H), 6.50 [m, 3H, NHC(NH_2)=N], 7.13 (m, 1H, Ph), 7.14 (d, 1H, *J* = 5 Hz, 1-H), 7.28-7.35 (m, 4H, Ph); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) *δ* (ppm): 13.2, 18.8, 20.3 [3CH_3 (Pbf)], 26.9 [C_γ (Arg)], 29.4 [2CH_3 (Pbf)], 30.6 [C_β (Arg)], 36.4 [1-CH_2], 41.7 (C_8), 41.8 [C_δ (Arg)], 44.0 [CH_2 (Pbf)], 52.8 (C_{8a}), 54.4 (C_5), 55.4 (C_1), 87.8, 118.1, 126.0 [3C (Pbf)], 128.1, 130.2, 130.4 [5CH (Ph)], 133.5, 136.6 [2C (Pbf)], 139.4 [C (Pbf) and C (Ph)], 158.0 [C (NHC(NH_2)=N)], 159.5 [C (Pbf)], 162.3 (C_3), 170.6 (C_6); ES-MS *m/z* 597.5 $[M+1]^+$; Anal. calcd. for $C_{30}H_{40}N_6O_5S$: C, 60.38; H, 6.76; N, 14.08. Found: C, 60.12; H, 6.47; N, 14.20.

*4.4.4. (1*S,*5*S,*8a*S)*-5-(3-(2-((2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-guanidino)propyl)-1-phenylmethyl-3,6-dioxooctahydroimidazo[1,5-*a*]pyrazine [**(S)-9a**]*

Foam (111 mg, 60%); $[\alpha]_D^{20}$ -9.9 (*c* 1.2, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 10-100% gradient of solvent A in 30 min] t_R 21.31 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.44 [s, 6H, $2CH_3$ (Pbf)], 1.45 [m, 2H, γ-H (Arg)], 1.66 [m, 1H, β-H (Arg)], 1.97 [m, 1H, β-H (Arg)], 2.05 [s, 3H, CH_3 (Pbf)], 2.42 [m, 1H, δ-H (Arg)], 2.50 [s, 3H, CH_3 (Pbf)], 2.57 [s, 3H, CH_3 (Pbf)], 2.74 (ddd, 1H, *J* = 3.5, 5.5 and 12 Hz, 8-H), 2.87 (m, 1H, 1-CH_2), 2.99 [s, 2H, CH_2 (Pbf)], 3.07 (m, 2H, 8-H and 1-CH_2), 3.17 [m, 1H, δ-H (Arg)], 3.51 (dt, 1H, *J* = 3.5 and 10 Hz, 8a-H), 3.77 (ddd, 1H, *J* = 6, 7.5 and 10 Hz, 1-H), 3.86 (dd, 1H, *J* = 3 and 6 Hz, 5-H), 6.01 (m, 1H, 2-H), 6.30 [m, 1H, NHC(NH_2)=N], 6.60 [m, 2H, NHC(NH_2)=N], 7.13 (d, 1H, *J* = 5.5 Hz, 1-H), 7.21-7.38 (m, 5H, Ph); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 11.9, 17.5, 18.9 [$3CH_3$ (Pbf)], 24.2 [C_γ (Arg)], 27.7 [C_β (Arg)], 28.8 [$2CH_3$ (Pbf)], 40.2 [1-CH_2], 40.8 [C_δ (Arg)], 43.0 [CH_2 (Pbf)], 44.0 (C_8), 57.2 (C_5), 56.0 (C_1), 58.7 (C_{8a}), 86.3, 116.7, 124.6 [3C (Pbf)], 126.9, 128.8, 129.5 [5CH (Ph)], 132.1, 134.9 [2C (Pbf)], 137.4 [C (Ph)], 138.0 [C (Pbf)], 156.6 [C (NHC(NH_2)=N)], 158.2 [C (Pbf)], 160.3 (C_3), 169.6 (C_6); ES-MS *m/z* 597.6 $[M+1]^+$; Anal. calcd. for $C_{30}H_{40}N_6O_5S$: C, 60.38; H, 6.76; N, 14.08. Found: C, 60.45; H, 6.50; N, 13.93.

*4.5 General procedure for the N_4-alkylation of 2-oxopiperazines **6a**,**b** and **7a**. Synthesis of the 4-alkyl-2-oxopiperazines **(R)-** and **(S)-(10a,b-13a)***

DIEA (456 μL, 2.71 mmol) and benzyl bromide (541 μL, 2.71 mmol) or *tert*-butyl bromoacetate (540 μL, 2.71 mmol) were added under argon in four equal portions over 72 h to a solution of the corresponding 2-oxopiperazine **(R)-** and **(S)-6a**,**b** and **-7a** (0.66 mmol) in anhydrous CH_3CN (10 mL) at 60 ºC. After 4 days of stirring, the solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (100 mL). The solution was successively washed with H_2O (2×25 mL) and brine (25 mL), dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography, using MeOH in CH_2Cl_2 gradient as mobile phase to obtain the respective 4-benzyl-2-oxopiperazines **(R)-** and **(S)-10a**,**b-13a**.

*4.5.1. (5*R,*3*S)*-4-Benzyl-5-((*S)*-1-((*tert*-butoxycarbonyl)amino-2-(1-(*tert*-butoxycarbonyl)-indol-3-yl)ethyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [**(R)-10a**]*

Reddish solid (446 mg, 75%); $[\alpha]_D^{20}$ -48 (*c* 1.5, CH_2Cl_2); Mp: 106-108 ºC (EtOAc/hexane); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 7.35 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.29 (s, 9H, Boc), 1.42 [s, 6H, $2CH_3$ (Pbf)], 1.65 [s, 3H, Boc (Ind)], 1.82 [m, 4H, β-H and γ-H (Arg)], 2.02 [s, 3H, CH_3 (Pbf)], 2.49 [s, 3H, CH_3 (Pbf)], 2.57 [s, 3H, CH_3 (Pbf)], 2.88 (m, 1H, *CH*$_2$-Ind), 2.92 [s, 2H, CH_2 (Pbf)], 3.03 [m, 1H, 3-H], 3.06 [m, 2H, δ-H (Arg)], 3.49 (m, 1H, 5-H), 3.50 (m, 1H, 6-H), 3.61 [m, 3H, *CH*$_2$-Ind, 4-CH_2 (Bn) and 6-H], 4.20 [m, 2H, 5-CH and 4-CH_2 (Bn)], 6.10 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 6.47 [m, 3H, NHC(NH_2)=N], 6.97 (s, 1H, 1-H), 7.15 (t, 1H, *J* = 7.5 Hz, Ar), 7.28 (m, 4H, Ar), 7.51 (m, 2H, Ar), 7.53 (m, 2H, Ar), 8.12 (d, 1H, *J* = 7.5 Hz, Ind). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 12.5, 18.2, 19.5 [$3CH_3$ (Pbf)], 27.1 [C_γ (Arg)], 28.2 [*CH*$_2$-Ind and $2CH_3$ (Pbf)], 28.3 [$3CH_3$ (Boc)], 28.5 [C_β (Arg)], 28.7 [$3CH_3$ (Boc)], 41.3 [C_δ (Arg)], 41.5 (C_6), 43.6 [CH_2 (Pbf)], 51.4 [C_5-*CH*], 51.8 [4-CH_2 (Bn)], 55.4 (C_5), 61.9 (C_3), 78.8, 83.9 [2C (2Boc)], 86.9 [C (Pbf)], 115.8 [CH (Ar)], 117.4 [C (Pbf)], 118.8 [C (Ar)], 119.9, 123.2, 124.4, 124.9 [4CH (Ar)], 125.3 [C (Pbf)], 128.1, 129.3, 129.8 [5CH (Ar)], 132.0 [C (Ar)], 132.8, 135.6 [2C (Pbf)], 136.4 [C (Ar)], 138.7 [C (Pbf)], 139.6 [C (Ar)], 150.3, 156.6 [2CO (2Boc)], 157.4 [C (NHC(NH_2)=N)], 158.9 [C (Pbf)], 172.1 (C_2); ES-MS *m/z* 901.1 $[M+1]^+$; Anal. calcd. for $C_{48}H_{65}N_7O_8S$: C, 64.05; H, 7.28; N, 10.89. Found: C, 64.23; H, 7.05; N, 11.13.

*4.5.2. (5*S,*3*S*)-4-Benzyl-5-((*S*)-1-((*tert-*butoxycarbonyl)amino-2-(1-(*tert-*butoxycarbonyl)-indol-3-yl)ethyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-guanidino)propyl)-2-oxopiperazine [**(S)-10a**]*

Reddish solid (357 mg, 60%); $[\alpha]_D^{20}$ -14 (*c* 1.1, CH_2Cl_2); Mp: 112-114 ºC (EtOAc/hexane); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 7.32 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.33 (s, 9H, Boc), 1.43 [s, 6H, $2CH_3$ (Pbf)], 1.44 [m, 2H, γ-H (Arg)], 1.55 [m, 3H, β-H (Arg)], 1.66 [s, 3H, Boc (Ind)], 2.03 [s, 3H, CH_3 (Pbf)], 2.47 [s, 3H, CH_3 (Pbf)], 2.55 [s, 3H, CH_3 (Pbf)], 2.86 (m, 1H, *CH*$_2$-Ind), 2.94 [s, 2H, CH_2 (Pbf)], 2.97 [m, 1H, δ-H (Arg)], 3.06 [m, 2H, δ-H (Arg) and 3-H], 3.14 (m, 1H, 5-H), 3.16 [m, 3H, *CH*$_2$-Ind], 3.47 (m, 1H, 6-H), 3.60 (m, 1H, 6-H), 3.76 [d, 1H, *J* = 14 Hz, 4-CH_2 (Bn)], 4.05 [d, 1H, *J* = 14 Hz, 4-CH_2 (Bn)], 4.14 [m, 2H, 5-CH], 6.18 (d, 1H, *J* = 9 Hz, *NH*Boc), 6.40 [m, 3H, NHC(NH_2)=N], 7.17 (s, 1H, 1-H), 7.24-7.55 (m, 8H, Ar), 7.64 (d, 1H, *J* = 7.5 Hz, Ar), 8.13 (d, 1H, *J* = 7.5 Hz, Ind); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 12.6, 18.2, 19.5 [$3CH_3$ (Pbf)], 26.6 [*CH*$_2$-Ind], 27.6 [C_γ (Arg)], 28.3 [$3CH_3$ (Boc)],

28.6 [2CH_3 (Pbf)], 28.7 [3CH_3 (Boc)], 31.4 [C_β (Arg)], 40.3 (C_6), 41.3 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 53.5 [C_5-*CH*], 62.3 [4-CH_2 (Bn)], 63.4 (C_5), 63.5 (C_3), 78.9, 84.0 [2C (2Boc)], 86.9 [C (Pbf)], 115.9 [CH (Ar)], 117.4 [C (Pbf)], 118.9 [C (Ar)], 120.0, 123.2, 124.1, 125.0 [4CH (Ar)], 125.2 [C (Pbf)], 128.1, 129.1, 130.3 [5CH (Ar)], 131.9 [C (Ar)], 132.8, 135.7 [2C (Pbf)], 136.3 [C (Ar)], 138.7 [C (Pbf)], 140.0 [C (Ar)], 150.3, 156.7 [2CO (2Boc)], 157.3 [C (NHC(NH_2)=N)], 158.9 [C (Pbf)], 173.4 (C_2); ES-MS *m/z* 901.1 $[M+1]^+$; Anal. calcd. for $C_{48}H_{65}N_7O_8S$: C, 64.05; H, 7.28; N, 10.89. Found: C, 63.87; H, 7.03; N, 11.24.

*4.5.3. (5*R,*3*S*)-4-Benzyl-5-((*S*)-1-((*tert-*butoxycarbonyl)amino-2-(1-(*tert-*butoxycarbonyl)-indol-3-yl)ethyl-3-(4-((*tert-*butoxycarbonyl)amino)butyl)-2-oxopiperazine [**(R)-10b**]*

Reddish solid (333 mg, 70%); $[\alpha]_D^{20}$ +0.9 (c 1.1, CH_2Cl_2); Mp: 76-78 ºC (EtOAc/hexane); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 70-100% gradient of solvent A in B, 30 min] t_R 11.27 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.29 [m, 2H, γ-H (Lys)], 1.30 (s, 9H, Boc), 1.34 [m, 2H, δ-H (Lys)], 1.36 [s, 9H, Boc (Lys)], 1.61 [m, 1H, β-H (Lys)], 1.66 [s, 9H, Boc (Ind)], 1.72 [m, 1H, β-H (Lys)], 1.83 [m, 1H, ε-H (Lys)], 2.88 (dd, 1H, *J* = 11 and 15 Hz, *CH*$_2$-Ind), 3.00 [m, 1H, ε-H (Lys)], 3.04 (m, 1H, 3-H), 3.49 (m, 1H, 5-H), 3.50 (m, 1H, 6-H), 3.60 [m, 2H, 4-CH_2 (Bn) and 6-H], 3.63 (m, 1H, *CH*$_2$-Ind), 4.22 [m, 1H, 4-CH_2 (Bn)], 4.23 (m, 1H, 5-CH), 5.80 [m, 1H, *NH*Boc (Lys)], 6.08 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 6.82 (s, 1H, 1-H), 7.18 (t, 1H, *J* = 7.5 Hz, Ar), 7.29 (m, 2H, Ar), 7.52 (m, 2H, Ar), 7.53 (m, 4H, Ar), 8.12 (d, 1H, *J* = 7.5 Hz, Ar). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 24.5 [C_γ (Lys)], 28.2 [*CH*$_2$-Ind], 28.3, 28.5, 28.7 [9CH_3 (3Boc)], 30.3 [C_δ (Lys)], 31.1 [C_β (Lys)], 41.1 [C_ε (Lys)], 41.5 (C_6), 51.3 [C_5-*CH*], 51.9 [4-CH_2 (Bn)], 55.4 (C_5), 62.2 (C_3), 78.8, 83.9 [3C (3Boc)], 115.8 [CH (Ar)], 118.8 [C (Ar)], 119.9, 123.2, 124.3, 124.9, 128.0, 129.3, 129.9 [9CH (Ar)], 132.0, 136.4, 139.7 [3C (Ar)], 150.3, 156.6 [3CO (3Boc)], 172.0 (C_2); ES-MS *m/z* 720.9 $[M+1]^+$; Anal. calcd. for $C_{40}H_{57}N_5O_7$: C, 66.73; H, 7.98; N, 9.73. Found: C, 66.51; H, 8.15; N, 9.95.

*4.5.4. (5*S,*3*S*)-4-Benzyl-5-((*S*)-1-((*tert-*butoxycarbonyl)amino-2-(1-(*tert-*butoxycarbonyl)-indol-3-yl)ethyl-3-(4-((*tert-*butoxycarbonyl)amino)butyl)-2-oxopiperazine [**(S)-10b**]*

Reddish solid (381 mg, 80%); $[\alpha]_D^{20}$ +13.6 (*c* 1.2, CH_2Cl_2); Mp: 84-86 ºC (EtOAc/hexane); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 70-100% gradient of solvent A in B 30 min] t_R 11.86 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.21 [m, 2H, γ-H (Lys)], 1.31 [m, 2H, δ-H (Lys)], 1.36 [m, 19H, Boc, Boc (Lys) and β-H (Lys)], 1.67 [s, 9H, Boc (Ind)], 1.68 [m, 1H, β-H (Lys)], 2.92 (m, 1H, *CH*$_2$-Ind), 2.94 [m, 2H, ε-H (Lys)], 3.07 (t, 1H,

J = 7 Hz, 3-H), 3.14 (m, 1H, 5-H), 3.24 (dd, 1H, *J* = 2.5 and 15 Hz, *CH*$_2$-Ind), 3.53 (m, 2H, 6-H), 3.80 [d, 1H, *J* = 14 Hz, 4-CH$_2$ (Bn)], 4.08 [d, 1H, *J* = 14 Hz, 4-CH$_2$ (Bn)], 4.17 (m, 1H, 5-CH), 5.82 [m, 1H, *NH*Boc (Lys)], 6.13 (d, 1H, *J* = 8.5 Hz, *NH*Boc), 7.03 (s, 1H, 1-H), 7.26 (m, 2H, Ar), 7.34 (m, 3H, Ar), 7.51 (m, 3H, Ar), 7.66 (d, 1H, *J* = 7.5 Hz, Ar), 8.14 (d, 1H, *J* = 7.5 Hz, Ar). ^{13}C NMR (100 MHz, (CD$_3$)$_2$CO) *δ* (ppm): 24.1 [C$_\gamma$ (Lys)], 26.6 [*CH*$_2$-Ind], 28.3, 28.7 [9CH$_3$ (3Boc)], 30.0 [C$_\delta$ (Lys)], 34.4 [C$_\beta$ (Lys)], 40.2 (C$_6$), 40.9 [C$_\varepsilon$ (Lys)], 52.9 [C$_5$-*CH*], 62.4 [4-CH$_2$ (Bn)], 63.9 (C$_5$), 64.0 (C$_3$), 78.2, 78.8, 84.0 [3C (3Boc)], 115.9 [CH (Ar)], 119.0 [C (Ar)], 120.0, 123.2, 124.0, 125.0, 128.0, 129.1, 130.2 [9CH (Ar)], 131.9, 136.4, 140.2 [3C (Ar)], 150.3, 156.6 [3CO (3Boc)], 173.2 (C$_2$); ES-MS *m/z* 720.9 [M+1]$^+$; Anal. calcd. for $C_{40}H_{57}N_5O_7$: C, 66.73; H, 7.98; N, 9.73. Found: C, 66.49; H, 8.21; N, 9.51.

*4.5.5. (5*R,*3*S*)-4-Benzyl-5-((*S*)-1-(*tert*-butoxycarbonyl)amino-2-phenylethyl)-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino) propyl)-2-oxopiperazine [**(R)-11a**]*

Reddish solid (401 mg, 80%); $[\alpha]_D^{20}$ -25 (*c* 1.0, CH_2Cl_2); Mp: 102-104 ºC (EtOAc/hexane); HPLC [Sunfire C$_{18}$ (4.6 × 150 mm, 3.5 μm), 60-100% gradient of solvent A in B, 30 min] t_R 16.05 min; ^{1}H NMR (400 MHz, (CD$_3$)$_2$CO) *δ* (ppm): 1.28 (s, 9H, Boc), 1.45 [s, 6H, 2CH$_3$ (Pbf)], 1.73 [m, 4H, β-H and γ-H (Arg)], 2.05 [s, 3H, CH$_3$ (Pbf)], 2.51 [s, 3H, CH$_3$ (Pbf)], 2.58 [s, 3H, CH$_3$ (Pbf)], 2.78 (dd, 1H, *J* = 11 and 14 Hz, *CH*$_2$-Ph), 2.98 [s, 2H, CH$_2$ (Pbf)], 3.00 [m, 2H, δ-H (Arg)], 3.06 [m, 1H, 3-H], 3.40 (m, 1H, 5-H), 3.44 (m, 1H, 6-H), 3.50 (m, 1H, *CH*$_2$-Ph), 3.54 [m, 1H, 4-CH$_2$ (Bn)], 3.60 (m, 1H, 6-H), 4.10 (m, 1H, 5-CH), 4.21 [d, 1H, *J* = 13.5 Hz, 4-CH$_2$ (Bn)], 6.01 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 6.48 [m, 3H, NHC(NH$_2$)=N], 6.94 (s, 1H, 1-H), 7.14-7.52 (m, 10H, Ar); ^{13}C NMR (100 MHz, (CD$_3$)$_2$CO) *δ* (ppm): 12.5, 18.2, 19.5 [3CH$_3$ (Pbf)], 27.1 [C$_\gamma$ (Arg)], 28.3 [C$_\beta$ (Arg)], 28.5 [3CH$_3$ (Boc)], 28.7 [2CH$_3$ (Pbf)], 38.6 [*CH*$_2$-Ph], 41.2 (C$_6$), 41.4 [C$_\delta$ (Arg)], 43.6 [CH$_2$ (Pbf)], 51.7 [4-CH$_2$ (Bn)], 53.1 [C$_5$-*CH*], 55.4 (C$_5$), 61.9 (C$_3$), 78.7 [C (Boc)], 86.9, 117.5, 125.3 [3C (Pbf)], 126.7, 128.1, 128.9, 129.3, 129.9, 130.2 [10CH (Ar)], 132.8, 135.6, 138.7 [3C (Pbf)], 139.7 [C (Ar)], 140.3 [C (Ar)], 156.5 [CO (Boc)], 157.3 [C (NHC(NH$_2$)=N)], 158.9 [C (Pbf)], 172.0 (C$_2$); ES-MS *m/z* 761.6 [M+1]$^+$; Anal. calcd. for $C_{41}H_{56}N_6O_6S$: C, 64.71; H, 7.42; N, 11.04. Found: C, 64.48; H, 7.65; N, 11.25.

*4.5.6. (5*S,*3*S*)-4-Benzyl-5-((*S*)-1-(*tert-*butoxycarbonyl)amino-2-phenylethyl)-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino) propyl)-2-oxopiperazine [**(S)-11a**]*

Reddish solid (351 mg, 70%); $[\alpha]_D^{20}$ +21 (*c* 1.0, CH_2Cl_2); Mp: 100-102 ºC (EtOAc/hexane); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 60-100% gradient of solvent A in B, 30 min] t_R 16.01 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.27 [m, 2H, γ-H (Arg)], 1.30 (s, 9H, Boc), 1.43 [s, 6H, $2CH_3$ (Pbf)], 1.59 [m, 2H, β-H (Arg)], 2.04 [s, 3H, CH_3 (Pbf)], 2.48 [s, 3H, CH_3 (Pbf)], 2.54 [s, 3H, CH_3 (Pbf)], 2.68 (dd, 1H, *J* = 11 and 13.5 Hz, *CH*$_2$-Ph), 2.93 [m, 1H, δ-H (Arg)], 2.97 [s, 2H, CH_2 (Pbf)], 3.00 (m, 1H, 5-H), 3.01 [m, 1H, δ-H (Arg)], 3.06 [m, 1H, 3-H], 3.10 (m, 1H, *CH*$_2$-Ph), 3.38 (t, 1H, *J* = 13 Hz, 6-H), 3.53 (dt, 1H, *J* = 6 and 13 Hz, 6-H), 3.72 [d, 1H, *J* = 14 Hz, 4-CH_2 (Bn)], 3.99 (m, 1H, 5-CH), 4.06 [m, 1H, 4-CH_2 (Bn)], 6.17 (d, 1H, *J* = 9 Hz, *NH*Boc), 6.44 [m, 3H, NHC(NH_2)=N], 7.12-7.48 (m, 11H, 1-H and Ar); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 11.9, 17.5, 18.8 [$3CH_3$ (Pbf)], 25.9 [C_γ (Arg)], 28.0 [$3CH_3$ (Boc)], 28.2 [$2CH_3$ (Pbf)], 30.9 [C_β (Arg)], 36.1 [*CH*$_2$-Ph], 39.5 (C_6), 40.5 [C_δ (Arg)], 42.9 [CH_2 (Pbf)], 54.8 (C_5-*CH*), 61.6 [4-CH_2 (Bn)], 62.7 (C_5), 63.1 (C_3), 78.2 [C (Boc)], 86.2, 116.7, 124.6 [3C (Pbf)], 126.1, 127.4, 128.3, 128.4, 129.3, 129.6 [10CH (Ar)], 132.1, 134.9, 138.0 [3C (Pbf)], 139.5 [C (Ar)], 139.7 [C (Ar)], 155.9 [CO (Boc)], 156.6 [C (NHC(NH_2)=N)], 158.2 [C (Pbf)], 172.6 (C_2); ES-MS *m/z* 761.6 $[M+1]^+$; Anal. calcd. for $C_{41}H_{56}N_6O_6S$: C, 64.71; H, 7.42; N, 11.04. Found: C, 64.53; H, 7.21; N, 11.31.

*4.5.7. (5*R,*3*S*)-5-((*S*)-1-((*tert-*Butoxycarbonyl)amino-2-(1-(*tert-*butoxycarbonyl)-indol-3-yl)ethyl-4-(*tert-*butoxycarbonyl)methyl -3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [**(R)-12a**]*

Reddish solid (397 mg, 65%); $[\alpha]_D^{20}$ +19 (*c* 1.4, CH_2Cl_2); Mp: 102-104 ºC (EtOAc/hexane); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 7.48 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.28 (s, 9H, Boc), 1.42 [s, 6H, $2CH_3$ (Pbf)], 1.47 (s, 9H, ^{t}Bu), 1.66 [s, 3H, Boc (Ind)], 1.80 [m, 4H, β-H and γ-H (Arg)], 2.03 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.58 [s, 3H, CH_3 (Pbf)], 2.80 (m, 1H, *CH*$_2$-Ind), 2.93 [s, 2H, CH_2 (Pbf)], 3.20 [m, 1H, 3-H], 3.21 (m, 1H, 4-CH_2), 3.33 [m, 2H, δ-H (Arg)], 3.40 (m, 1H, 5-H), 3.49 (m, 2H, 6-H), 3.60 (m, 1H, *CH*$_2$-Ind), 3.64 (m, 1H, 4-CH_2), 3.92 (m, 1H, 5-CH), 6.07 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 6.53 [m, 3H, NHC(NH_2)=N], 6.92 (s, 1H, 1-H), 7.24 (t, 1H, *J* = 7.5 Hz, Ind), 7.30 (t, 1H, *J* = 7.5 Hz, Ind), 7.51 (s, 1H, Ind), 7.64 (d,

1H, *J* = 7.5 Hz, Ind), 8.12 (d, 1H, *J* = 7.5 Hz, Ind); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 12.5, 18.2, 19.5 [$3CH_3$ (Pbf)], 27.4 (*CH_2*-Ind), 27.8 [C_γ (Arg)], 28.3 [$3CH_3$ (Boc)], 28.5 [C_β (Arg) and $3CH_3$ (^{t}Bu)], 28.7 [$3CH_3$ (Boc) and $2CH_3$ (Pbf)], 41.6 [C_δ (Arg)], 41.8 (C_6), 43.6 [CH_2 (Pbf)], 51.7 (C_5-*CH*), 51.9 (4-CH_2), 55.6 (C_5), 64.9 (C_3), 78.8 [C (Boc)], 81.6 [C (^{t}Bu)], 83.9 [C (Boc)], 86.9 [C (Pbf)], 115.8 [CH (Ind)], 117.4 [C (Pbf)], 118.9 [C (Ind)], 120.0, 123.1, 124.5, 124.9 [4CH (Ind)], 125.3 [C (Pbf)], 131.9 [C (Ind)], 132.8, 135.6 [2C (Pbf)], 136.4 [C (Ind)], 138.7 [C (Pbf)], 150.3, 156.6 [2CO (2Boc)], 157.4 [C (NHC(NH_2)=N)], 158.9 [C (Pbf)], 171.0 (CO_2), 171.8 (C_2); ES-MS *m/z* 925.2 $[M+1]^+$; Anal. calcd. for $C_{47}H_{69}N_7O_{10}S$: C, 61.08; H, 7.53; N, 10.61. Found: C, 61.35; H, 7.29; N, 10.48.

*4.5.8. (5*S*,3*S*)-5-((*S*)-1-((*tert*-Butoxycarbonyl)amino-2-(1-(*tert*-butoxycarbonyl)-indol-3-yl)ethyl-4-(*tert*-butoxycarbonyl)methyl -3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [**(S)-12a**]*

Reddish solid (305 mg, 50%); $[\alpha]_D^{20}$ +9.0 (*c* 1.2, CH_2Cl_2); Mp: 120-122 ºC (EtOAc/hexane); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 7.19 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.32 (s, 9H, Boc), 1.43 [s, 6H, $2CH_3$ (Pbf)], 1.44 (s, 9H, ^{t}Bu), 1.66 [s, 3H, Boc (Ind)], 1.77 [m, 4H, β-H and γ-H (Arg)], 2.03 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.57 [s, 3H, CH_3 (Pbf)], 2.79 (dd, 1H, *J* = 10 and 14 Hz, *CH_2*-Ind), 2.96 [s, 2H, CH_2 (Pbf)], 3.20 (m, 1H, *CH_2*-Ind), 3.21 (m, 1H, 5-H), 3.24 [m, 2H, δ-H (Arg)], 3.32 [m, 1H, 3-H], 3.46 (m, 1H, 6-H), 3.55 (m, 2H, 4-CH_2 and 6-H), 3.66 (d, 1H, *J* = 18 Hz, 4-CH_2), 4.05 (m, 1H, 5-CH), 6.24 (d, 1H, *J* = 8.5 Hz, *NH*Boc), 6.50 [m, 3H, NHC(NH_2)=N], 7.15 (s, 1H, 1-H), 7.24 (t, 1H, *J* = 7.5 Hz, Ind), 7.31 (t, 1H, *J* = 7.5 Hz, Ind), 7.51 (s, 1H, Ind), 7.64 (d, 1H, *J* = 7.5 Hz, Ind), 8.12 (d, 1H, *J* = 7.5 Hz, Ind); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 12.5, 18.2, 19.5 [$3CH_3$ (Pbf)], 25.5 (*CH_2*-Ind), 26.4 [C_γ (Arg)], 28.3 [$6CH_3$ (Boc and ^{t}Bu)], 28.6 [$2CH_3$ (Pbf)], 28.7 [$3CH_3$ (Boc)], 31.6 [C_β (Arg)], 39.9 [C_6], 41.6 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 52.1 (C_5-*CH*), 56.9 (4-CH_2), 60.9 (C_5), 64.6 (C_3), 78.8 [C (Boc)], 81.7 [C (^{t}Bu)], 84.0 [C (Boc)], 86.9 [C (Pbf)], 115.9 [CH (Ind)], 117.4 [C (Pbf)], 119.1 [C (Ind)], 120.0, 123.1, 124.1, 125.0 [4CH (Ind)], 125.6 [C (Pbf)], 131.8 [C (Ind)], 132.8, 135.7 [2C (Pbf)], 136.4 [C (Ind)], 138.7 [C (Pbf)], 150.3, 156.5 [2CO (2Boc)], 157.3 [C (NHC(NH_2)=N)], 158.9 [C (Pbf)], 171.3 (CO_2 and C_2); ES-MS *m/z* 925.1 $[M+1]^+$; Anal. calcd. for $C_{47}H_{69}N_7O_{10}S$: C, 61.08; H, 7.53; N, 10.61. Found: C, 61.29; H, 7.75; N, 10.40.

*4.5.9. (5*R,*3*S*)-5-((*S*)-1-((*tert-*Butoxycarbonyl)amino-2-(1-(*tert-*butoxycarbonyl)-indol-3-yl)ethyl-4-(*tert-*butoxycarbonyl)methyl -3-(4-((*tert-*butoxycarbonyl)amino)butyl)-2-oxopiperazine [(**R**)-12b]*

Reddish solid (368 mg, 75%); $[\alpha]_D^{20}$ +3 (*c* 1.7, CH_2Cl_2); Mp: 78-80 ºC (EtOAc/hexane); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 70-100% gradient of solvent A in b, 30 min] t_R 11.54 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.29 (s, 9H, Boc), 1.36 [s, 9H, Boc (Lys)], 1.49 (s, 9H, ^{t}Bu), 1.59 [m, 2H, γ-H (Lys)], 1.60 [m, 2H, δ-H (Lys)], 1.67 [s, 9H, Boc (Ind)], 1.79 [m, 1H, β-H (Lys)], 1.80 [m, 1H, β-H (Lys)], 2.78 (dd, 1H, *J* = 11 and 15 Hz, *CH*$_2$-Ind), 3.12 [m, 2H, ε-H (Lys)], 3.19 (m, 2H, 3-H and 4-CH_2), 3.39 (m, 1H, 5-H), 3.41 (m, 2H, 6-H), 3.64 (d, 1H, *J* = 16 Hz, 4-CH_2), 3.66 (dd, 1H, *J* = 2 and 15 Hz, *CH*$_2$-Ind), 3.94 (m, 1H, 5-CH), 5.93 [m, 1H, *NH*Boc (Lys)], 6.07 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 6.84 (s, 1H, 1-H), 7.23 (t, 1H, *J* = 7.5 Hz, Ind), 7.30 (t, 1H, *J* = 7.5 Hz, Ind), 7.51 (s, 1H, Ind), 7.64 (d, 1H, *J* = 7.5 Hz, Ind), 8.13 (d, 1H, *J* = 7.5 Hz, Ind). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 24.7 [C_γ (Lys)], 27.8 (*CH*$_2$-Ind), 28.3 [6CH_3 (Boc and ^{t}Bu)], 28.5, 28.7 [6CH_3 (2Boc)], 30.4 [C_δ (Lys)], 31.1 [C_β (Lys)], 41.2 [C_ε (Lys)], 41.8 (C_6), 51.9 (C_5-*CH*), 52.1 (4-CH_2), 55.8 (C_5), 65.2 (C_3), 78.2, 78.8 [2C (2Boc)], 81.5[C (^{t}Bu)], 83.9 [C (Boc)], 115.8 [CH (Ind)], 118.9 [C (Ind)], 120.0, 123.1, 124.3, 124.9 [4CH (Ind)], 132.0, 136.4 [2C (Ind)], 150.3, 156.6 [3CO (3Boc)], 171.0 (CO_2), 171.8 (C_2); ES-MS *m/z* 744.9 $[M+1]^+$; Anal. calcd. for $C_{39}H_{61}N_5O_9$: C, 62.97; H, 8.26; N, 9.41. Found: C, 63.21; H, 8.15; N, 9.24.

*4.5.10. (5*S,*3*S*)-5-((*S*)-1-((*tert-*Butoxycarbonyl)amino-2-(1-(*tert-*butoxycarbonyl)-indol-3-yl)ethyl-4-(*tert-*butoxycarbonyl)methyl -3-(4-((*tert-*butoxycarbonyl)amino)butyl)-2-oxopiperazine [(**S**)-12b]*

Reddish solid (295 mg, 60%); $[\alpha]_D^{20}$ +10 (*c* 1.2, CH_2Cl_2); Mp: 80-82 ºC (EtOAc/hexane); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 70-100% gradient of solvent A in B, 30 min] t_R 12.04 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.34 (s, 9H, Boc), 1.37 [s, 9H, Boc (Lys)], 1.45 (s, 9H, ^{t}Bu), 1.53 [m, 2H, γ-H (Lys)], 1.56 [m, 2H, δ-H (Lys)], 1.66 [s, 9H, Boc (Ind)], 1.77 [m, 2H, β-H (Lys)], 2.80 (m, 1H, *CH*$_2$-Ind), 3.07 [m, 2H, ε-H (Lys)], 3.23 (m, 1H, *CH*$_2$-Ind), 3.27 (m, 1H, 5-H), 3.30 (m, 1H, 3-H), 3.50 (m, 2H, 6-H), 3.57 (d, 1H, *J* = 18 Hz, 4-CH_2), 3.68 (d, 1H, *J* = 18 Hz, 4-CH_2), 4.07 (m, 1H, 5-CH), 5.93 [m, 1H, *NH*Boc (Lys)], 6.21 (d, 1H, *J* = 8.5 Hz, *NH*Boc), 7.00 (s, 1H, 1-H), 7.26 (t, 1H, *J* = 7.5 Hz, Ind), 7.31 (t, 1H, *J* = 7.5 Hz, Ind), 7.52 (s, 1H, Ind), 7.67 (d, 1H, *J* = 7.5 Hz, Ind), 8.13 (d, 1H, *J* = 7.5 Hz, Ind). ^{13}C

NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 23.8 [C_γ (Lys)], 25.6 (*CH_2*-Ind), 28.3 [6CH_3 (Boc and ^{t}Bu)], 28.6 [6CH_3 (2Boc)], 30.4 [C_δ (Lys)], 34.5 [C_β (Lys)], 39.9 (C_6), 41.1 [C_ε (Lys)], 52.0 (C_5-*CH*), 57.0 (4-CH_2), 60.1 (C_5), 65.3 (C_3), 78.2, 78.8 [2C (2Boc)], 81.7 [C (^{t}Bu)], 84.0 [C (Boc)], 115.9 [CH (Ind)], 119.1 [C (Ind)], 119.6, 123.3, 124.1, 125.0 [4CH (Ind)], 131.8, 136.4 [2C (Ind)], 150.3, 156.4 [3CO (3Boc)], 171.3 (CO_2), 172.6 (C_2); ES-MS *m/z* 744.9 $[M+1]^+$; Anal. calcd. for $C_{39}H_{61}N_5O_9$: C, 62.97; H, 8.26; N, 9.41. Found: C, 62.71; H, 8.05; N, 9.63.

*4.5.11. (5*R,*3*S*)-5-((*S*)-1-(*tert-*Butoxycarbonyl)amino-2-phenylethyl)- 4-(*tert-*butoxycarbonyl)-methyl -3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino) propyl)-2-oxopiperazine [**(R)-13a**]*

Reddish solid (363 mg, 70%); $[\alpha]_D^{20}$ -19 (*c* 1.0, CH_2Cl_2); Mp: 100-102 ºC (EtOAc/hexane); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 60-100% gradient of solvent A in B, 30 min] t_R 15.95 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.26 (s, 9H, Boc), 1.44 [s, 6H, 2CH_3 (Pbf)], 1.46 (s, 9H, ^{t}Bu), 1.72 [m, 2H, γ-H (Arg)], 1.95 [m, 2H, β-H (Arg)], 2.04 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.58 [s, 3H, CH_3 (Pbf)], 2.67 (dd, 1H, *J* = 11 and 14 Hz, *CH_2*-Ph), 2.97 [s, 2H, CH_2 (Pbf)], 3.15 (m, 2H, 3-H and 4-CH_2), 3.30 [m, 2H, δ-H (Arg)], 3.32 (m, 1H, 5-H), 3.39 (m, 2H, 6-H), 3.50 (dd, 1H, *J* = 3 and 14 Hz, *CH_2*-Ph), 3.61 (d, 1H, *J* = 16 Hz, 4-CH_2), 3.85 (m, 1H, 5-CH), 5.97 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 6.55 [m, 3H, NHC(NH_2)=N], 6.90 (s, 1H, 1-H), 7.13-7.19 (m, 5H, Ph); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 12.5, 18.2, 19.5 [3CH_3 (Pbf)], 27.3 [C_γ (Arg)], 28.3 [3CH_3 (^{t}Bu)], 28.5 [3CH_3 (Boc)], 28.7 [C_β (Arg) and 2CH_3 (Pbf)], 38.2 (*CH_2*-Ph), 41.6 [C_δ (Arg)], 41.8 [C_6], 43.6 [CH_2 (Pbf)], 51.7 (4-CH_2), 54.0 (C_5-*CH*), 55.5 (C_5), 64.9 (C_3), 78.7 [C (Boc)], 81.4 [C (^{t}Bu)], 86.9, 117.4, 125.3 [3C (Pbf)], 126.7, 128.8, 130.3 [5CH (Ph)], 132.8, 135.6, 138.7 [3C (Pbf)], 140.5 [C (Ph)], 156.4 [CO (Boc)], 157.4 [C (NHC(NH_2)=N)], 158.9 [C (Pbf)], 171.0 (CO_2), 171.8 (C_2); ES-MS *m/z* 785.7 $[M+1]^+$; Anal. calcd. for $C_{40}H_{60}N_6O_8S$: C, 61.20; H, 7.70; N, 10.71. Found: C, 61.48; H, 7.40; N, 11.00.

*4.5.12. (5*S,*3*S*)-5-((*S*)-1-(*tert-*Butoxycarbonyl)amino-2-phenylethyl)- 4-(*tert-*butoxycarbonyl)-methyl -3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino) propyl)-2-oxopiperazine [**(S)-13a**]*

Reddish solid (363 mg, 70%); $[\alpha]_D^{20}$ +11 (*c* 1.2, CH_2Cl_2); Mp: 98-100 ºC (EtOAc/hexane); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 60-100% gradient of solvent A in B, 30 min] t_R

14.98 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.29 (s, 9H, Boc), 1.44 [s, 6H, $2CH_3$ (Pbf)], 1.45 (s, 9H, ^{t}Bu), 1.70 [m, 2H, γ-H (Arg)], 1.72 [m, 2H, β-H (Arg)], 2.05 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.58 [s, 3H, CH_3 (Pbf)], 2.63 (dd, 1H, *J* = 11 and 14 Hz, *CH*$_2$-Ph), 2.98 [s, 2H, CH_2 (Pbf)], 3.10 (dd, 1H, *J* = 3 and 14 Hz, *CH*$_2$-Ph), 3.19 (m, 1H, 5-H), 3.24 [m, 2H, δ-H (Arg)], 3.31 [m, 1H, 3-H], 3.38 (m, 1H, 6-H), 3.50 (m, 1H, 6-H), 3.51 (m, 1H, 4-CH_2), 3.65 (d, 1H, *J* = 18 Hz, 4-CH_2), 3.92 (m, 1H, 5-CH), 6.16 (d, 1H, *J* = 8.5 Hz, *NH*Boc), 6.55 [m, 3H, NHC(NH_2)=N], 7.10-7.21 (m, 2H, 1-H and Ph), 7.22-7.31 (m, 4H, Ph); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 13.2, 18.9, 20.1 [$3CH_3$ (Pbf)], 27.0 [C_γ (Arg)], 28.9 [$3CH_3$ (^{t}Bu)], 29.0 [$3CH_3$ (Boc)], 29.2 [$2CH_3$ (Pbf)], 32.3 [C_β (Arg)], 36.4 (*CH*$_2$-Ph), 40.6 (C_6), 42.3 [C_δ (Arg)], 44.3 [CH_2 (Pbf)], 54.8 (C_5-*CH*), 57.4 (4-CH_2), 61.4 (C_5), 65.3 (C_3), 79.4 [C (Boc)], 82.4 [C (^{t}Bu)], 87.6, 118.1, 125.9 [3C (Pbf)], 127.5, 129.7, 130.6 [5CH (Ph)], 133.5, 136.3, 139.4 [3C (Pbf)], 141.3 [C (Ph)], 156.9 [CO (Boc)], 158.0 [C (NHC(NH_2)=N)], 159.5 [C (Pbf)], 171.2 (CO_2), 173.5 (C_2); ES-MS *m/z* 785.7 $[M+1]^+$; Anal. calcd. for $C_{40}H_{60}N_6O_8S$: C, 61.20; H, 7.70; N, 10.71. Found: C, 61.01; H, 7.62; N, 10.94.

4.6. General procedure for the benzylation of the 4-alkyl-2-oxopiperazines ***10a-13a****. Synthesis of the 4-alkyl-1-benzyl-2-oxopiperazines* ***(R)-*** *and* ***(S)-(14a****,* ***17a****,* ***20a*** *and* ***23a****), and* ***(R)-*** *and* ***(S)-(16a****,* ***19a****,* ***22a*** *and* ***25a****)*

NaH (60% suspension in mineral oil, 24 mg, 1.02 mmol) and benzyl bromide (152 µL, 0.51 mmol) were added in three equal portions over 2 h to a solution of the corresponding 2-oxopiperazine **10a-13a** (0.34 mmol) in anhydrous mixture THF/DMF (9:1, 10 mL) under argon at 0 ºC. After 1h of stirring, the crude reaction mixture was diluted with EtOAc (100 mL) and the excess of NaH was hydrolysed by addition of H_2O (20 mL). The aqueous layer was extracted with EtOAc (2×50 mL) and the organic extracts were dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography, using MeOH in CH_2Cl_2 gradient as mobile phase to give the respective 4-alkyl-1-benzyl-2-oxopiperazines **14a**, **17a**, **20a** and **23a** in 40-57 % and the respective derivative benzylated at the guanidino group **16a**, **19a**, **22a** and **25a** as (Z/E)-isomeric mixtures in 11-20 % yield. The compounds were dissolved in CH_3CN/H_2O (1:2, 2 mL) and the solutions were lyophilized to obtain amorphous solids.

*4.6.1. (5*R*,3*S*)-1,4-Dibenzyl-5-((*S*)-1-((*tert*-butoxycarbonyl)amino-2-(1-(*tert*-butoxy-carbonyl)-indol-3-yl)ethyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [**(R)-14a**]*

Amorphous solid (135 mg, 40%); $[\alpha]_D^{20}$ -12 (*c* 1.2, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 13.05 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.24 (s, 9H, Boc), 1.42 [s, 6H, $2CH_3$ (Pbf)], 1.64 [s, 3H, Boc (Ind)], 1.85 [m, 4H, β-H and γ-H (Arg)], 2.03 [s, 3H, CH_3 (Pbf)], 2.49 [s, 3H, CH_3 (Pbf)], 2.56 [s, 3H, CH_3 (Pbf)], 2.83 (m, 1H, *CH_2*-Ind), 2.92 [s, 2H, CH_2 (Pbf)], 3.09 [m, 2H, δ-H (Arg)], 3.16 [m, 1H, 3-H], 3.39 (m, 1H, 6-H), 3.53 [m, 1H, *CH_2*-Ind], 3.54 [m, 1H, 4-CH_2 (Bn)], 3.58 (m, 2H, 5-H and 6-H), 4.14 [m, 1H, 5-CH], 4.17 [d, 1H, *J* = 13.5 Hz, 4-CH_2 (Bn)], 4.51 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 4.79 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 5.98 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 6.47 [m, 3H, NHC(NH_2)=N], 7.07-7.55 (m, 14H, Ar), 8.10 (d, 1H, *J* = 8 Hz, Ar). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 12.6, 18.2, 19.5 [$3CH_3$ (Pbf)], 27.2 (*CH_2*-Ind), 27.9 [C_γ (Arg)], 28.3 [$3CH_3$ (Boc)], 28.5 [$2CH_3$ (Pbf)], 28.6 [$3CH_3$ (Boc)], 28.8 [C_β (Arg)], 41.3 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 45.6 (C_6), 50.2 [1-CH_2 (Bn)], 51.3 (C_5-*CH*), 51.9 [4-CH_2 (Bn)], 55.7 (C_5), 62.2 (C_3), 78.9, 83.9 [2C (2Boc)], 86.9 [C (Pbf)], 115.8 [CH (Ar)], 117.4 [C (Pbf)], 118.7 [C (Ar)], 119.9, 123.2, 124.4, 124.9 [4CH (Ar)], 125.3 [C (Pbf)], 128.2, 128.8, 129.3, 129.5, 129.8 [10CH (Ar)], 131.9 [C (Ar)], 132.8, 135.6 [2C (Pbf)], 136.3 [C (Ar)], 138.7 [2C (Pbf and Ar)], 139.4 [C (Ar)], 150.3, 156.5 [2CO (2Boc)], 157.3 [C (NHC(NH_2)=N)], 158.9 [C (Pbf)], 170.8 (C_2); ES-MS *m/z* 991.1 $[M+1]^+$; Anal. calcd. for $C_{55}H_{71}N_7O_8S$: C, 66.71; H, 7.23; N, 9.90. Found: C, 66.98; H, 7.41; N, 9.54.

*4.6.2. (5*S*,3*S*)-1,4-Dibenzyl-5-((*S*)-1-((*tert*-butoxycarbonyl)amino-2-(1-(*tert*-butoxy-carbonyl)-indol-3-yl)ethyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [**(S)-14a**]*

Amorphous solid (175 mg, 52%); $[\alpha]_D^{20}$ +22 (c 1.1, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 13.87 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.29 (s, 9H, Boc), 1.42 [s, 6H, $2CH_3$ (Pbf)], 1.66 [s, 3H, Boc (Ind)], 1.73 [m, 4H, β-H and γ-H (Arg)], 2.03 [s, 3H, CH_3 (Pbf)], 2.48 [s, 3H, CH_3 (Pbf)], 2.56 [s, 3H, CH_3 (Pbf)], 2.78 (m, 1H, *CH_2*-Ind), 2.94 [s, 2H, CH_2 (Pbf)], 3.00 [m, 1H, δ-H (Arg)], 3.07 [m, 1H, δ-H (Arg)], 3.13 (m, 1H, *CH_2*-Ind), 3.15 (m, 1H, 5-H), 3.26 [m, 1H, 3-H], 3.53 (m, 2H, 6-H), 3.81 [d, 1H, *J* = 14 Hz, 4-CH_2 (Bn)], 4.04 [d, 1H, *J* = 14 Hz, 4-CH_2

(Bn)], 4.10 (m, 1H, 5-CH), 4.53 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 4.69 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 6.13 (d, 1H, *J* = 9 Hz, *NH*Boc), 6.39 [m, 3H, NHC(NH_2)=N], 7.14-7.36 (m, 11H, Ar), 7.39-7.50 (m, 2H, Ar), 7.58 (d, 1H, *J* = 7.5 Hz, Ar), 8.12 (d, 1H, *J* = 7.5 Hz, Ar). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 12.6, 18.2, 19.5 [$3CH_3$ (Pbf)], 26.3 (*CH_2*-Ind), 26.8 [C_γ (Arg)], 28.3 [$3CH_3$ (Boc)], 28.6 [$2CH_3$ (Pbf)], 28.7 [$3CH_3$ (Boc)], 31.9 [C_β (Arg)], 41.3 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 45.2 (C_6), 49.9 [1-CH_2 (Bn)], 53.2 (C_5-*CH*), 62.0 [4-CH_2 (Bn)], 63.2 (C_5), 63.7 (C_3), 79.0, 84.0 [2C (2Boc)], 86.9 [C (Pbf)], 115.9 [CH (Ar)], 117.4 [C (Pbf)], 118.8 [C (Ar)], 120.0, 123.2, 124.1, 125.0 [4CH (Ar)], 125.2 [C (Pbf)], 128.2, 128.7, 129.1, 129.5, 130.4 [10CH (Ar)], 131.9 [C (Ar)], 132.8, 135.7 [2C (Pbf)], 136.3 [C (Ar)], 138.6 [C (Ar)], 138.7 [C (Pbf)], 139.7 [C (Ar)], 150.3, 156.7 [2CO (2Boc)], 157.2 [C (NHC(NH_2)=N)], 158.9 [C (Pbf)], 171.8 (C_2); ES-MS *m/z* 991.2 $[M+1]^+$; Anal. calcd. for $C_{55}H_{71}N_7O_8S$: C, 66.71; H, 7.23; N, 9.90. Found: C, 66.85; H, 7.52; N, 9.61.

*4.6.3. (5*R,*3*S*)-1,4-Dibenzyl-5-((*S*)-1-((*tert-*butoxycarbonyl)amino-2-(1-(*tert-*butoxy-carbonyl)-indol-3-yl)ethyl-3-(3-(3-benzyl-2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [(**R**)-16a]*

Amorphous solid (2:1 isomeric mixture, 55 mg, 15%); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 20.20 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm) Major isomer: 1.25 (s, 9H, Boc), 1.40 [s, 3H, CH_3 (Pbf)], 1.45 [s, 3H, CH_3 (Pbf)], 1.63 [s, 3H, Boc (Ind)], 1.83 [m, 2H, γ-H (Arg)], 1.92 [m, 2H, β-H (Arg)], 2.00 [s, 3H, CH_3 (Pbf)], 2.45 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.81 [s, 2H, CH_2 (Pbf)], 2.90 (m, 1H, *CH_2*-Ind), 3.07 [m, 2H, δ-H (Arg)], 3.14 [m, 1H, 3-H], 3.38 (m, 1H, 6-H), 3.55 [m, 2H, 5-H and 4-CH_2 (Bn)], 3.57 (m, 1H, 6-H), 3.58 [m, 1H, *CH_2*-Ind], 4.20 [m, 1H, 4-CH_2 (Bn)], 4.22 [m, 1H, 5-CH], 4.43 [t, 2H, *J* = 5.5 Hz, CH_2 (NHC(NH*Bn*)=N)], 4.54 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 4.78 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 5.99 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 7.02-7.57 [m, 21H, Ar and *NH*C(*NH*Bn)=N], 8.11 (d, 1H, *J* = 8 Hz, Ar). Minor isomer: 1.22 (s, 9H, Boc), 1.38 [s, 3H, CH_3 (Pbf)], 1.45 [s, 3H, CH_3 (Pbf)], 1.63 [s, 3H, Boc (Ind)], 1.83 [m, 2H, γ-H (Arg)], 1.92 [m, 2H, β-H (Arg)], 1.99 [s, 3H, CH_3 (Pbf)], 2.45 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.83 (m, 1H, *CH_2*-Ind), 2.93 [s, 2H, CH_2 (Pbf)], 3.07 [m, 2H, δ-H (Arg)], 3.14 [m, 1H, 3-H], 3.38 (m, 1H, 6-H), 3.47 [m, 1H, *CH_2*-Ind], 3.55 [m, 2H, 5-H and 4-CH_2 (Bn)], 3.57 (m, 1H, 6-H), 4.20 [m, 1H, 4-CH_2 (Bn)], 4.22 [m, 1H, 5-CH], 4.48 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 4.64 [m, 2H, CH_2 (NHC(NH*Bn*)=N)], 4.81 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 5.94 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 7.02-7.57 [m, 21H, Ar and *NH*C(*NH*Bn)=N], 8.11 (d,

1H, *J* = 8 Hz, Ar); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm) Major isomer: 12.5, 18.3, 19.6 [$3CH_3$ (Pbf)], 25.6 (*CH_2*-Ind), 27.0 [C_γ (Arg)], 28.0 [C_β (Arg)], 28.3 [$3CH_3$ (Boc) and $2CH_3$ (Pbf)], 28.6 [$3CH_3$ (Boc)], 41.6 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 45.3 [CH_2 (NHC(NH*Bn*)=N)], 45.9 (C_6), 50.3 [1-CH_2 (Bn)], 51.4 (C_5-*CH*), 51.8 [4-CH_2 (Bn)], 55.7 (C_5), 61.9 (C_3), 78.8, 83.9 [2C (2Boc)], 86.9 [C (Pbf)], 115.8 [CH (Ar)], 117.6 [C (Pbf)], 118.7 [C (Ar)], 120.0, 123.2, 124.5, 124.9 [4CH (Ar)], 125.3 [C (Pbf)], 128.2, 128.8, 129.4, 129.5, 129.8 [15CH (Ar)], 131.9 [C (Ar)], 132.8, 135.4 [2C (Pbf)], 136.3 [C (Ar)], 138.7 [2C (Pbf and Ar)], 138.8 [C (Ar)], 139.3 [C (Ar)], 150.3, 156.0 [2CO (2Boc)], 156.5 [C (NH*C*(NHBn)=N)], 158.9 [C (Pbf)], 170.7 (C_2). Minor isomer: 12.5, 18.3, 19.5 [$3CH_3$ (Pbf)], 25.6 [*CH_2*-Ind], 27.0 [C_γ (Arg)], 28.0 [C_β (Arg)], 28.3 [$3CH_3$ (Boc) and $2CH_3$ (Pbf)], 28.6 [$3CH_3$ (Boc)], 41.6 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 45.9 (C_6), 50.3 [1-CH_2 (Bn)], 51.5 (C_5-*CH*), 51.8 [4-CH_2 (Bn) and CH_2 (NHC(NH*Bn*)=N)], 55.6 (C_5), 62.0 (C_3), 78.8, 83.9 [2C (2Boc)], 86.9 [C (Pbf)], 115.8 [CH (Ar)], 117.5 [C (Pbf)], 118.6 [C (Ar)], 119.9, 123.2, 124.5, 124.9 [4CH (Ar)], 125.3 [C (Pbf)], 128.2, 128.9, 129.2, 129.4, 129.5 [15CH (Ar)], 131.9 [C (Ar)], 132.8, 135.4 [2C (Pbf)], 136.3 [C (Ar)], 138.7 [2C (Pbf and Ar)], 138.8 [C (Ar)], 139.3 [C (Ar)], 150.3, 156.0 [2CO (2Boc)], 156.5 [C (NH*C*(NHBn)=N)], 158.9 [C (Pbf)], 170.7 (C_2); ES-MS *m/z* 1081.2 $[M+1]^+$; Anal. calcd. for $C_{62}H_{77}N_7O_8S$: C, 68.93; H, 7.18; N, 9.08. Found: C, 69.24; H, 7.32; N, 8.91.

*4.6.4. (5*S*,3*S*)-1,4-Dibenzyl-5-((*S*)-1-((*tert*-butoxycarbonyl)amino-2-(1-(*tert*-butoxy-carbonyl)-indol-3-yl)ethyl-3-(3-(3-benzyl-2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [**(S)-16a**]*

Amorphous solid (3:1 isomeric mixture, 55 mg, 15%); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 20.78 min (major isomer) and 21.64 min (minor isomer); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm) Major isomer: 1.29 (s, 9H, Boc), 1.42 [s, 3H, CH_3 (Pbf)], 1.50 [s, 3H, CH_3 (Pbf)], 1.64 [m, 2H, γ-H (Arg)], 1.66 [s, 3H, Boc (Ind)], 1.71 [m, 2H, β-H (Arg)], 2.01 [s, 3H, CH_3 (Pbf)], 2.42 [s, 3H, CH_3 (Pbf)], 2.49 [s, 3H, CH_3 (Pbf)], 2.79 (m, 1H, *CH_2*-Ind), 2.88 [s, 2H, CH_2 (Pbf)], 3.11 [m, 2H, δ-H (Arg)], 3.14 [m, 2H, 5-H], 3.15 [m, 1H, *CH_2*-Ind], 3.30 [m, 1H, 3-H], 3.53 (m, 2H, 6-H), 3.82 [d, 1H, *J* = 14 Hz, 4-CH_2 (Bn)], 4.07 [m, 1H, 4-CH_2 (Bn)], 4.13 [m, 1H, 5-CH], 4.38 [d, 2H, *J* = 5 Hz, CH_2 (NHC(NH*Bn*)=N)], 4.53 [m, 1H, 1-CH_2 (Bn)], 4.69 [m, 1H, 1-CH_2 (Bn)], 6.12 (d, 1H, *J* = 8.5 Hz, *NH*Boc), 7.02-7.37 [m, 16H, Ar and *NH*C(*NH*Bn)=N], 7.44 (m, 4H, Ar), 7.60 (m, 1H, Ar), 8.12 (d, 1H, *J* = 8 Hz, Ar). Minor isomer: 1.29 (s, 9H, Boc), 1.43 [s, 3H, CH_3

(Pbf)], 1.45 [s, 3H, CH_3 (Pbf)], 1.64 [m, 5H, γ-H (Arg) and Boc (Ind)], 1.71 [m, 2H, β-H (Arg)], 2.02 [s, 3H, CH_3 (Pbf)], 2.46 [s, 3H, CH_3 (Pbf)], 2.54 [s, 3H, CH_3 (Pbf)], 2.79 (m, 1H, *CH_2*-Ind), 2.97 [s, 2H, CH_2 (Pbf)], 3.11 [m, 2H, δ-H (Arg)], 3.14 [m, 2H, 5-H], 3.15 [m, 1H, *CH_2*-Ind], 3.27 [m, 1H, 3-H], 3.53 (m, 2H, 6-H), 3.81 [d, 1H, *J* = 14 Hz, 4-CH_2 (Bn)], 4.04 [m, 1H, 4-CH_2 (Bn)], 4.13 [m, 1H, 5-CH], 4.59 [m, 1H, CH_2 (NHC(NH*Bn*)=N)], 4.50 [m, 1H, 1-CH_2 (Bn)], 4.60 [m, 1H, CH_2 (NHC(NH*Bn*)=N)], 4.63 [m, 1H, 1-CH_2 (Bn)], 6.07 (d, 1H, *J* = 8.5 Hz, *NH*Boc), 7.02-7.37 [m, 16H, Ar and *NH*C(*NH*Bn)=N], 7.43 (m, 4H, Ar), 7.56 (m, 1H, Ar), 8.12 (d, 1H, *J* = 8 Hz, Ar); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) *δ* (ppm) Major isomer: 12.6, 18.3, 19.6 [3CH_3 (Pbf)], 26.2 (*CH_2*-Ind), 26.4 [C_γ (Arg)], 28.3 [3CH_3 (Boc)], 28.6 [2CH_3 (Pbf)], 28.7 [3CH_3 (Boc)], 30.1 [C_β (Arg)], 41.6 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 44.9 (C_6), 45.3 [CH_2 (NHC(NH*Bn*)=N)], 49.9 [1-CH_2 (Bn)], 52.7 (C_5-*CH*), 61.9 [4-CH_2 (Bn)], 63.5 (C_3 and C_5), 78.9, 84.0 [2C (2Boc)], 86.9 [C (Pbf)], 115.9 [CH (Ar)], 117.5 [C (Pbf)], 118.9 [C (Ar)], 120.0, 123.2, 124.0, 125.0 [4CH (Ar)], 125.3 [C (Pbf)], 128.1, 128.7, 129.1, 129.5, 130.3 [15CH (Ar)], 131.8 [C (Ar)], 132.8, 135.9 [2C (Pbf)], 136.3 [C (Ar)], 138.4 [C (Ar)], 138.7 [C (Pbf], 139.6 [2C (Ar)], 150.3, 155.9 [2CO (2Boc)], 156.5 [C (NH*C*(NHBn)=N)], 158.9 [C (Pbf)], 171.7 (C_2). Minor isomer: 12.6, 18.2, 19.4 [3CH_3 (Pbf)], 26.2 (*CH_2*-Ind), 26.4 [C_γ (Arg)], 28.1 [3CH_3 (Boc)], 28.6 [2CH_3 (Pbf)], 28.7 [3CH_3 (Boc)], 30.2 [C_β (Arg)], 41.6 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 44.9 (C_6), 49.9 [1-CH_2 (Bn)], 51.0 [CH_2 (NHC(NH*Bn*)=N)], 52.7 (C_5-*CH*), 61.9 [4-CH_2 (Bn)], 63.5 (C_5), 64.0 (C_3), 78.9, 84.0 [2C (2Boc)], 87.3 [C (Pbf)], 115.9 [CH (Ar)], 117.5 [C (Pbf)], 118.9 [C (Ar)], 120.0, 123.2, 124.0, 125.0 [4CH (Ar)], 125.3 [C (Pbf)], 128.1, 128.7, 129.2, 129.4, 130.3 [15CH (Ar)], 131.8 [C (Ar)], 132.8, 136.1 [2C (Pbf)], 136.3 [C (Ar)], 138.4 [C (Ar)], 138.7 [C (Pbf], 139.6 [2C (Ar)], 150.3, 155.9 [2CO (2Boc)], 156.5 [C (NH*C*(NHBn)=N)], 158.9 [C (Pbf)], 171.7 (C_2); ES-MS *m/z* 1081.2 $[M+1]^+$; Anal. calcd. for $C_{62}H_{77}N_7O_8S$: C, 68.93; H, 7.18; N, 9.08. Found: C, 69.14; H, 7.02; N, 9.27.

*4.6.5. (5*R,*3*S*)-1,4-Dibenzyl-5-((*S*)-1-(*tert*-butoxycarbonyl)amino-2-phenylethyl)-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino) propyl)-2-oxopiperazine [**(R)-17a**]*

Amorphous solid (139 mg, 48%); $[\alpha]_D^{20}$ -12 (*c* 1.0, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 7.14 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) *δ* (ppm): 1.22 (s, 9H, Boc), 1.43 [s, 6H, 2CH_3 (Pbf)], 1.81 [m, 4H, β-H and γ-H (Arg)], 2.04 [s, 3H, CH_3 (Pbf)], 2.48 [s, 3H, CH_3 (Pbf)], 2.57 [s, 3H, CH_3 (Pbf)], 2.71 (dd, 1H,

J = 11 and 14 Hz, *CH*$_2$-Ph), 2.96 [s, 2H, CH$_2$ (Pbf)], 3.05 [m, 2H, δ-H (Arg)], 3.12 [m, 1H, 3-H], 3.32 (dd, 1H, *J* = 4 and 12 Hz, 6-H), 3.48 (m, 2H, 5-H and *CH*$_2$-Ph), 3.50 [m, 1H, 4-CH$_2$ (Bn)], 3.57 (m, 1H, 6-H), 4.06 (ddd, 1H, *J* = 3.5, 10 and 13 Hz, 5-CH), 4.17 [d, 1H, *J* = 14.5 Hz, 4-CH$_2$ (Bn)], 4.47 [d, 1H, *J* = 14.5 Hz, 1-CH$_2$ (Bn)], 4.78 [d, 1H, *J* = 14.5 Hz, 1-CH$_2$ (Bn)], 5.90 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 6.50 [m, 3H, NHC(NH$_2$)=N], 7.09-7.45 (m, 15H, Ar); ^{13}C NMR (100 MHz, (CD$_3$)$_2$CO) *δ* (ppm): 11.9, 17.6, 18.8 [3CH$_3$ (Pbf)], 26.5 [C$_\gamma$ (Arg)], 27.6 [C$_\beta$ (Arg)], 27.8 [3CH$_3$ (Boc)], 28.1 [2CH$_3$ (Pbf)], 37.6 (*CH*$_2$-Ph), 40.6 [C$_\delta$ (Arg)], 42.9 [CH$_2$ (Pbf)], 45.0 (C$_6$), 49.5 [1-CH$_2$ (Bn)], 51.0 [4-CH$_2$ (Bn)], 52.4 (C$_5$-*CH*), 55.0 (C$_5$), 61.5 (C$_3$), 78.1 [C (Boc)], 86.3, 116.8, 124.6 [3C (Pbf)], 126.1, 127.5, 128.1, 128.2, 128.6, 128.8, 129.3, 129.5 [15CH (Ar)], 132.2, 134.9 [2C (Pbf)], 138.0 [C (Ar)], 138.1 [2C (Pbf and Ar)], 139.5 [C (Ar)], 155.7 [CO (Boc)], 156.6 [C (NHC(NH$_2$)=N)], 158.2 [C (Pbf)], 170.2 (C$_2$); ES-MS *m/z* 852.0 [M+1]$^+$; Anal. calcd. for C$_{48}$H$_{62}$N$_6$O$_6$S: C, 67.74; H, 7.34; N, 9.87. Found: C, 67.51; H, 7.65; N, 9.63.

*4.6.6. (5*S,*3*S*)-1,4-Dibenzyl-5-((*S*)-1-(*tert*-butoxycarbonyl)amino-2-phenylethyl)-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino) propyl)-2-oxopiperazine [**(S)-17a**]*

Amorphous solid (130 mg, 45%); [α]$_D^{20}$ +21 (*c* 1.1, CH$_2$Cl$_2$); HPLC [Sunfire C$_{18}$ (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 6.96 min; ^{1}H NMR (400 MHz, (CD$_3$)$_2$CO) *δ* (ppm): 1.26 (s, 9H, Boc), 1.43 [s, 6H, 2CH$_3$ (Pbf)], 1.56 [m, 2H, γ-H (Arg)], 1.67 [m, 2H, β-H (Arg)], 2.04 [s, 3H, CH$_3$ (Pbf)], 2.49 [s, 3H, CH$_3$ (Pbf)], 2.56 [s, 3H, CH$_3$ (Pbf)], 2.60 (m, 1H, *CH*$_2$-Ph), 2.96 [s, 2H, CH$_2$ (Pbf)], 2.99 [m, 1H, δ-H (Arg)], 3.04 (m, 1H, *CH*$_2$-Ph), 3.05 [m, 1H, δ-H (Arg)], 3.07 (m, 2H, 5-H), 3.23 [m, 1H, 3-H], 3.45 (m, 2H, 6-H), 3.77 [m, 1H, *J* = 14 Hz, 4-CH$_2$ (Bn)], 3.97 (m, 1H, 5-CH), 4.03 [d, 1H, *J* = 14 Hz, 4-CH$_2$ (Bn)], 4.49 [d, 1H, *J* = 15 Hz, 1-CH$_2$ (Bn)], 4.69 [d, 1H, *J* = 15 Hz, 1-CH$_2$ (Bn)], 6.09 (d, 1H, *J* = 9 Hz, *NH*Boc), 6.41 [m, 3H, NHC(NH$_2$)=N], 7.11-7.29 (m, 15H, Ar); ^{13}C NMR (100 MHz, (CD$_3$)$_2$CO) *δ* (ppm): 12.6, 18.2, 19.5 [3CH$_3$ (Pbf)], 26.6 [C$_\gamma$ (Arg)], 28.6 [3CH$_3$ (Boc)], 28.7 [2CH$_3$ (Pbf)], 31.9 [C$_\beta$ (Arg)], 36.5 (*CH*$_2$-Ph), 41.2 [C$_\delta$ (Arg)], 43.6 [CH$_2$ (Pbf)], 45.1 (C$_6$), 49.8 [1-CH$_2$ (Bn)], 55.5 (C$_5$-*CH*), 62.0 [4-CH$_2$ (Bn)], 63.4 (C$_5$), 63.8 (C$_3$), 78.9 [C (Boc)], 86.9, 117.4, 125.3 [3C (Pbf)], 126.8, 128.2, 128.7, 128.9, 129.2, 129.5, 130.0, 130.4 [15CH (Ar)], 132.8, 135.7 [2C (Pbf)], 138.5 [C (Ar)], 138.7 [C (Pbf)], 139.9 [C (Ar)], 140.3 [C (Ar)], 156.5 [CO (Boc)], 157.2 [C (NHC(NH$_2$)=N)], 158.9 [C (Pbf)], 171.8 (C$_2$); ES-MS

m/z 852.0 $[M+1]^+$; Anal. calcd. for $C_{48}H_{62}N_6O_6S$: C, 67.74; H, 7.34; N, 9.87. Found: C, 67.86; H, 7.61; N, 10.12.

*4.6.7. (5*R*,3*S*)-1,4-Dibenzyl-5-((*S*)-1-(*tert*-butoxycarbonyl)amino-2-phenylethyl)-3-(3-(3-benzyl-2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino) propyl)-2-oxopiperazine [**(R)-19a**]*

Amorphous solid (2.5:1 isomeric mixture, 45 mg, 15%); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 13.14 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm) Major isomer: 1.21 (s, 9H, Boc), 1.41 [s, 3H, CH_3 (Pbf)], 1.45 [s, 3H, CH_3 (Pbf)], 1.80 [m, 4H, β-H and γ-H (Arg)], 2.01 [s, 3H, CH_3 (Pbf)], 2.45 [s, 3H, CH_3 (Pbf)], 2.51 [s, 3H, CH_3 (Pbf)], 2.74 (dd, 1H, *J* = 11 and 14 Hz, *CH*$_2$-Ph), 2.89 [s, 2H, CH_2 (Pbf)], 3.05 [m, 2H, δ-H (Arg)], 3.15 [m, 1H, 3-H], 3.33 (dd, 1H, *J* = 4 and 12 Hz, 6-H), 3.50 (m, 1H, 5-H), 3.52 [m, 1H, 4-CH_2 (Bn)], 3.56 (m, 1H, *CH*$_2$-Ph), 3.57 (m, 1H, 6-H), 4.12 (m, 1H, 5-CH), 4.18 [d, 1H, *J* = 13.5 Hz, 4-CH_2 (Bn)], 4.42 [m, 2H, CH_2 (NHC(NH*Bn*)=N)], 4.49 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 4.79 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 5.89 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 7.04-7.57 [m, 22H, Ar and *NHC*(*NH*Bn)=N]. Minor isomer: 1.22 (s, 9H, Boc), 1.43 [s, 3H, CH_3 (Pbf)], 1.44 [s, 3H, CH_3 (Pbf)], 1.80 [m, 4H, β-H and γ-H (Arg)], 2.01 [s, 3H, CH_3 (Pbf)], 2.46 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.66 (m, 1H, *CH*$_2$-Ph), 2.96 [s, 2H, CH_2 (Pbf)], 3.05 [m, 2H, δ-H (Arg)], 3.15 [m, 1H, 3-H], 3.30 (m, 1H, 6-H), 3.41 (dd, 1H, *J* = 3 and 14 Hz, *CH*$_2$-Ph), 3.50 (m, 1H, 5-H), 3.51 [m, 1H, 4-CH_2 (Bn)], 3.59 (m, 1H, 6-H), 4.10 [m, 1H, 4-CH_2 (Bn)], 4.12 (m, 1H, 5-CH), 4.49 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 4.60 [m, 2H, CH_2 (NHC(NH*Bn*)=N)], 4.81 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 5.86 (d, 1H, *J* = 10 Hz, *NH*Boc), 7.04-7.57 [m, 22H, Ar and *NHC*(*NH*Bn)=N]; ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm) Major isomer: 11.9, 17.6, 18.9 [3CH_3 (Pbf)], 26.2 [C_γ (Arg)], 27.6 [C_β (Arg) and 3CH_3 (Boc)], 27.8 [2CH_3 (Pbf)], 37.6 (*CH*$_2$-Ph), 40.5 [C_δ (Arg)], 42.9 [CH_2 (Pbf)], 45.1 [C_6 and CH_2 (NHC(NH*Bn*)=N)], 49.6 [1-CH_2 (Bn)], 51.0 [4-CH_2 (Bn)], 52.6 (C_5-*CH*), 55.0 (C_5), 61.4 (C_3), 78.0 [C (Boc)], 86.3, 116.9, 124.6 [3C (Pbf)], 127.5, 128.1, 128.3, 128.7, 128.8, 129.2, 129.6 [20CH (Ar)], 132.2, 134.9 [2C (Pbf)], 138.0 [C (Ar)], 138.1 [C (Ar)], 138.7 [C (Pbf)], 138.9 [C (Ar)], 139.5 [C (Ar)], 155.3 [C (NH*C*(NHBn)=N)], 155.6 [CO (Boc)], 158.3 [C (Pbf)], 170.1 (C_2). Minor isomer: 11.9, 17.6, 18.9 [3CH_3 (Pbf)], 26.2 [C_γ (Arg)], 27.6 [C_β (Arg) and 3CH_3 (Boc)], 27.8 [2CH_3 (Pbf)], 37.7 (*CH*$_2$-Ph), 40.5 [C_δ (Arg)], 42.9 [CH_2 (Pbf)], 44.7 (C_6), 49.6 [1-CH_2 (Bn)], 50.8 [CH_2 (NHC(NH*Bn*)=N)], 51.0 [4-CH_2 (Bn)], 52.4 (C_5-*CH*), 55.0 (C_5), 61.1 (C_3), 78.0 [C (Boc)], 86.3, 116.9, 124.6 [3C (Pbf)],

127.5, 128.2, 128.3, 128.5, 129.2, 129.5 [20CH (Ar)], 132.2, 134.9 [2C (Pbf)], 138.0 [C (Ar)], 138.1 [C (Ar)], 138.7 [C (Pbf)], 138.9 [C (Ar)], 139.5 [C (Ar)], 155.3 [C (NH*C*(NHBn)=N)], 155.7 [CO (Boc)], 158.3 [C (Pbf)], 170.1 (C_2); ES-MS *m/z* 942.2 $[M+1]^+$; Anal. calcd. for $C_{55}H_{68}N_6O_6S$: C, 70.18; H, 7.28; N, 8.93. Found: C, 69.92; H, 7.47; N, 8.67.

*4.6.8. (5*S,*3*S*)-1,4-Dibenzyl-5-((*S*)-1-(*tert*-butoxycarbonyl)amino-2-phenylethyl)-3-(3-(3-benzyl-2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino) propyl)-2-oxopiperazine [**(S)-19a**]*

Amorphous solid (5:3 isomeric mixture, 35 mg, 11%); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 12.49 (major isomer) and 13.19 (minor isomer) min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm) Major isomer: 1.26 (s, 9H, Boc), 1.44 [s, 6H, $2CH_3$ (Pbf)], 1.64 [m, 4H, β-H and γ-H (Arg)], 2.01 [s, 3H, CH_3 (Pbf)], 2.43 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.62 (m, 1H, *CH*$_2$-Ph), 2.94 [s, 2H, CH_2 (Pbf)], 3.08 [m, 3H, 5-H and δ-H (Arg)], 3.09 (m, 1H, *CH*$_2$-Ph), 3.26 [m, 1H, 3-H], 3.46 (m, 2H, 6-H), 3.78 [d, 1H, *J* = 14 Hz, 4-CH_2 (Bn)], 4.06 [d, 1H, *J* = 14 Hz, 4-CH_2 (Bn)], 4.08 (m, 1H, 5-CH), 4.40 [d, 2H, *J* = 5.5 Hz, CH_2 (NHC(NH*Bn*)=N)], 4.49 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 4.68 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 6.05 (d, 1H, *J* = 8.5 Hz, *NH*Boc), 7.02-7.52 [m, 22H, Ar and *NH*C(*NH*Bn)=N]. Minor isomer: 1.25 (s, 9H, Boc), 1.45 [s, 6H, $2CH_3$ (Pbf)], 1.64 [m, 4H, β-H and γ-H (Arg)], 2.01 [s, 3H, CH_3 (Pbf)], 2.47 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.62 (m, 1H, *CH*$_2$-Ph), 2.97 [s, 2H, CH_2 (Pbf)], 3.08 [m, 3H, 5-H and δ-H (Arg)], 3.09 (m, 1H, *CH*$_2$-Ph), 3.26 [m, 1H, 3-H], 3.46 (m, 2H, 6-H), 3.77 [d, 1H, *J* = 14 Hz, 4-CH_2 (Bn)], 4.06 [d, 1H, *J* = 14 Hz, 4-CH_2 (Bn)], 4.08 (m, 1H, 5-CH), 4.49 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 4.58 [m, 2H, CH_2 (NHC(NH*Bn*)=N)], 4.64 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 6.05 (d, 1H, *J* = 8.5 Hz, *NH*Boc), 7.02-7.52 [m, 22H, Ar and *NH*C(*NH*Bn)=N]; ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm) Major isomer: 12.5, 18.7, 19.6 [$3CH_3$ (Pbf)], 26.4 [C_γ (Arg)], 28.6 [$3CH_3$ (Boc)], 29.3 [$2CH_3$ (Pbf)], 32.1 [C_β (Arg)], 36.3 (*CH*$_2$-Ph), 41.6 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 44.9 [C_6 and CH_2 (NHC(NH*Bn*)=N)], 49.8 [1-CH_2 (Bn)], 54.7 (C_5-*CH*), 61.7 [4-CH_2 (Bn)], 63.6 (C_3 and C_5), 78.9 [C (Boc)], 86.9, 117.4, 125.3 [3C (Pbf)], 126.8, 128.2, 128.7, 129.0, 129.1, 129.2, 129.5, 129.9, 130.3 [20CH (Ar)], 132.8, 135.6 [2C (Pbf)], 138.4 [C (Ar)], 138.7 [C (Pbf)], 139.5 [C (Ar)], 139.8 [C (Ar)], 139.9 [C (Ar)], 155.9 [C (NH*C*(NHBn)=N)], 156.3 [CO (Boc)], 158.9 [C (Pbf)], 171.8 (C_2). Minor isomer: 12.5, 18.7, 19.6 [$3CH_3$ (Pbf)], 26.4 [C_γ (Arg)], 28.6 [$3CH_3$ (Boc)], 29.3 [$2CH_3$ (Pbf)], 32.1 [C_β (Arg)], 36.3 (*CH*$_2$-Ph), 41.6 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 44.9 (C_6), 49.9 [1-CH_2 (Bn)], 51.0 [CH_2

(NHC(NH*Bn*)=N)], 54.7 (C_5-*CH*), 61.7 [4-CH_2 (Bn)], 63.6 (C_3 and C_5), 78.9 [C (Boc)], 86.9, 117.4, 125.3 [3C (Pbf)], 126.8, 127.9, 128.2, 128.5, 128.8, 129.4, 129.9, 130.2 [20CH (Ar)], 132.8, 135.6 [2C (Pbf)], 138.4 [C (Ar)], 138.7 [C (Pbf)], 139.5 [C (Ar)], 139.8 [C (Ar)], 139.9 [C (Ar)], 156.5 [C (NH*C*(NHBn)=N)], 156.3 [CO (Boc)], 158.9 [C (Pbf)], 171.8 (C_2); ES-MS *m/z* 942.2 $[M+1]^+$; Anal. calcd. for $C_{55}H_{68}N_6O_6S$: C, 70.18; H, 7.28; N, 8.93. Found: C, 70.35; H, 7.03; N, 9.22.

*4.6.9. (5*R,*3*S*)-1-Benzyl-5-((*S*)-1-((*tert-*butoxycarbonyl)amino-2-(1-(*tert-*butoxycarbonyl)-indol-3-yl)ethyl-4-(*tert-*butoxycarbonyl)methyl -3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [(**R**)-20a]*

Amorphous solid (172 mg, 50%); $[\alpha]_D^{20}$ +3.1 (*c* 1.1, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 12.48 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.22 (s, 9H, Boc), 1.42 [s, 6H, $2CH_3$ (Pbf)], 1.46 (s, 9H, ^{t}Bu), 1.65 [s, 3H, Boc (Ind)], 1.90 [m, 4H, β-H and γ-H (Arg)], 2.03 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.58 [s, 3H, CH_3 (Pbf)], 2.78 (m, 1H, *CH*$_2$-Ind), 2.93 [s, 2H, CH_2 (Pbf)], 3.19 (d, 1H, *J* = 16 Hz, 4-CH_2), 3.29 (m, 1H, 6-H), 3.32 [m, 1H, 3-H], 3.34 [m, 2H, δ-H (Arg)], 3.41 (m, 1H, 6-H), 3.50 (m, 1H, 5-H), 3.56 (m, 1H, *CH*$_2$-Ind), 3.64 (d, 1H, *J* = 16 Hz, 4-CH_2), 3.90 (m, 1H, 5-CH), 4.37 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 4.84 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 5.97 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 6.52 [m, 3H, NHC(NH_2)=N], 7.20 (t, 1H, *J* = 7.5 Hz, Ar), 7.25-7.38 (m, 6H, Ar), 7.49 (s, 1H, Ar), 7.58 (d, 1H, *J* = 7.5 Hz, Ar), 8.11 (d, 1H, *J* = 7.5 Hz, Ar). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 12.6, 18.2, 19.5 [$3CH_3$ (Pbf)], 27.5 [*CH*$_2$-Ind and C_γ (Arg)], 28.2 [$3CH_3$ (^{t}Bu)], 28.3 [$3CH_3$ (Boc)], 28.5 [C_β (Arg) and $2CH_3$ (Pbf)], 28.7 [$3CH_3$ (Boc)], 41.6 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 46.1 (C_6), 50.2 [1-CH_2 (Bn)], 51.7 (4-CH_2), 51.9 (C_5-*CH*), 55.8 (C_5), 65.2 (C_3), 78.8 [C (Boc)], 81.7 [C (^{t}Bu)], 83.9 [C (Boc)], 86.9 [C (Pbf)], 115.8 [CH (Ar)], 117.4 [C (Pbf)], 118.9 [C (Ar)], 120.0, 123.1, 124.5, 124.9 [4CH (Ar)], 125.3 [C (Pbf)], 127.1, 128.7, 129.4 [5CH (Ar)], 131.9 [C (Ar)], 132.8, 135.6 [2C (Pbf)], 136.3 [C (Ar)], 138.6 [C (Pbf)], 138.7 [C (Ar)], 150.3, 156.5 [2CO (2Boc)], 157.4 [C (NHC(NH_2)=N)], 158.9 [C (Pbf)], 170.6 (C_2), 170.9 (CO_2); ES-MS *m/z* 1015.2 $[M+1]^+$; Anal. calcd. for $C_{54}H_{75}N_7O_{10}S$: C, 63.94; H, 7.45; N, 9.67. Found: C, 64.22; H, 7.63; N, 9.36.

*4.6.10. (5*S,*3*S*)-1-Benzyl-5-((*S*)-1-((*tert-*butoxycarbonyl)amino-2-(1-(*tert-*butoxycarbonyl)-indol-3-yl)ethyl-4-(*tert-*butoxycarbonyl)methyl -3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [(**S**)-20a]*

Amorphous solid (197 mg, 57%); $[\alpha]_D^{20}$ +20 (*c* 1.1, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 13.67 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.27 (s, 9H, Boc), 1.43 [s, 6H, $2CH_3$ (Pbf)], 1.45 (s, 9H, ^{t}Bu), 1.65 [s, 3H, Boc (Ind)], 1.76 [m, 2H, γ-H (Arg)], 1.80 [m, 2H, β-H (Arg)], 2.04 [s, 3H, CH_3 (Pbf)], 2.49 [s, 3H, CH_3 (Pbf)], 2.58 [s, 3H, CH_3 (Pbf)], 2.68 (dd, 1H, *J* = 11 and 15 Hz, *CH*$_2$-Ind), 2.95 [s, 2H, CH_2 (Pbf)], 3.13 (m, 1H, *CH*$_2$-Ind), 3.26 [m, 2H, δ-H (Arg)], 3.28 (m, 1H, 5-H), 3.49 (m, 3H, 3-H and 6-H), 3.58 (d, 1H, *J* = 18 Hz, 4-CH_2), 3.68 (d, 1H, *J* = 18 Hz, 4-CH_2), 4.01 (m, 1H, 5-CH), 4.59 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 4.69 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 6.15 (d, 1H, *J* = 8 Hz, *NH*Boc), 6.49 [m, 3H, NHC(NH_2)=N], 7.18-7.38 (m, 7H, Ar), 7.47 (s, 1H, Ar), 7.58 (d, 1H, *J* = 7.5 Hz, Ar), 8.11 (d, 1H, *J* = 7.5 Hz, Ar). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 12.6, 18.2, 19.5 [$3CH_3$ (Pbf)], 25.3 (*CH*$_2$-Ind), 26.5 [C_γ (Arg)], 28.3 [$3CH_3$ (^{t}Bu) and $3CH_3$ (Boc)], 28.6 [$2CH_3$ (Pbf)], 28.7 [$3CH_3$ (Boc)], 32.2 [C_β (Arg)], 41.7 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 44.9 (C_6), 50.0 [1-CH_2 (Bn)], 52.0 (C_5-*CH*), 56.6 (4-CH_2), 60.9 (C_5), 65.0 (C_3), 78.9 [C (Boc)], 81.7 [C (^{t}Bu)], 84.0 [C (Boc)], 86.9 [C (Pbf)], 115.9 [CH (Ar)], 117.4 [C (Pbf)], 119.0 [C (Ar)], 119.9, 123.3, 124.1, 125.0 [4CH (Ar)], 125.3 [C (Pbf)], 128.1, 128.8, 129.4 [5CH (Ar)], 131.7 [C (Ar)], 132.8, 135.7 [2C (Pbf)], 136.3, 138.5 [2C (Ar)], 138.7 [C (Pbf)], 150.3, 156.4 [2CO (2Boc)], 157.3 [C (NHC(NH_2)=N)], 158.9 [C (Pbf)], 171.0 (C_2), 171.3 (CO_2); ES-MS *m/z* 1015.2 $[M+1]^+$; Anal. calcd. for $C_{54}H_{75}N_7O_{10}S$: C, 63.94; H, 7.45; N, 9.67. Found: C, 63.76; H, 7.69; N, 9.92.

*4.6.11. (5*R,*3*S*)-1-Benzyl-5-((*S*)-1-((*tert-*butoxycarbonyl)amino-2-(1-(*tert-*butoxycarbonyl)-indol-3-yl)ethyl-4-(*tert-*butoxycarbonyl)methyl -3-(3-(3-benzyl-2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [(**R**)-22a]*

Amorphous solid (5:1 isomeric mixture, 60 mg, 16%); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 20.05 min (major isomer) and 19.34 min (minor isomer**)**; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm) Major isomer: 1.20 (s, 9H, Boc), 1.42 [s, 6H, $2CH_3$ (Pbf)], 1.46 (s, 9H, ^{t}Bu), 1.64 [s, 3H, Boc (Ind)], 1.90 [m, 4H, β-H and γ-H (Arg)], 2.00 [s, 3H, CH_3 (Pbf)], 2.44 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.79 (m, 1H, *CH*$_2$-Ind), 2.93 [s, 2H, CH_2 (Pbf)], 3.20 (d, 1H, *J* = 16 Hz, 4-CH_2), 3.30 (m, 1H, 6-H), 3.34 [m, 1H, 3-H], 3.38 [m, 1H, δ-H (Arg)], 3.41 (m, 1H, 6-H), 3.47 [m, 1H, δ-H (Arg)], 3.49 (m, 1H, 5-H), 3.57 (m, 1H, *CH*$_2$-Ind), 3.65 (d, 1H, *J* = 16 Hz, 4-CH_2), 3.90 (m, 1H, 5-CH), 4.38 [dd, 1H, *J* = 6 and 15 Hz, CH_2 (NHC(NH*Bn*)=N)], 4.40 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 4.46 [dd, 1H, *J* = 6 and 15 Hz, CH_2 (NHC(NH*Bn*)=N)], 4.83 [d, 1H, *J* = 14.5 Hz, 1-

CH_2 (Bn)], 5.96 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 7.09-7.40 [m, 14H, Ar and *NH*C(*NH*Bn)=N], 7.50 (s, 1H, Ar), 7.61 (d, 1H, *J* = 7.5 Hz, Ar), 8.11 (d, 1H, *J* = 7.5 Hz, Ar). Minor isomer: 1.23 (s, 9H, Boc), 1.43 [s, 6H, $2CH_3$ (Pbf)], 1.44 (s, 9H, ^{t}Bu), 1.66 [s, 3H, Boc (Ind)], 1.90 [m, 4H, β-H and γ-H (Arg)], 2.00 [s, 3H, CH_3 (Pbf)], 2.45 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.79 (m, 1H, *CH_2*-Ind), 2.95 [s, 2H, CH_2 (Pbf)], 3.22 (d, 1H, *J* = 16 Hz, 4-CH_2), 3.30 (m, 1H, 6-H), 3.34 [m, 1H, 3-H], 3.38 [m, 1H, δ-H (Arg)], 3.41 (m, 1H, 6-H), 3.47 [m, 1H, δ-H (Arg)], 3.49 (m, 1H, 5-H), 3.57 (m, 1H, *CH_2*-Ind), 3.65 (d, 1H, *J* = 16 Hz, 4-CH_2), 3.90 (m, 1H, 5-CH), 4.35 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 4.68 [d, 1H, *J* = 16 Hz, CH_2 (NHC(NH*Bn*)=N)], 4.77 [d, 1H, *J* = 16 Hz, CH_2 (NHC(NH*Bn*)=N)], 4.85 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 5.94 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 7.09-7.40 [m, 14H, Ar and *NH*C(*NH*Bn)=N], 7.47 (s, 1H, Ar), 7.55 (d, 1H, *J* = 7.5 Hz, Ar), 8.11 (d, 1H, *J* = 7.5 Hz, Ar); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) *δ* (ppm) Major isomer: 12.5, 18.3, 19.6 [$3CH_3$ (Pbf)], 27.2 (*CH_2*-Ind), 27.6 [C_γ (Arg)], 28.3 [$3CH_3$ (^{t}Bu)], 28.7 [$3CH_3$ (Boc)], 28.4 [C_β (Arg) and $2CH_3$ (Pbf)], 28.7 [$3CH_3$ (Boc)], 41.8 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 45.4 [CH_2 (NHC(NH*Bn*)=N)], 46.2 (C_6), 50.3 [1-CH_2 (Bn)], 51.6 (4-CH_2), 52.0 (C_5-*CH*), 55.5 (C_5), 65.0 (C_3), 78.8 [C (Boc)], 81.7 [C (^{t}Bu)], 83.9 [C (Boc)], 86.9 [C (Pbf)], 115.8 [CH (Ar)], 117.5 [C (Pbf)], 118.6 [C (Ar)], 120.0, 123.1, 124.7, 124.9 [4CH (Ar)], 125.3 [C (Pbf)], 128.1, 128.7, 129.5 [10CH (Ar)], 131.9 [C (Ar)], 132.8, 135.6 [2C (Pbf)], 136.4 [C (Ar)], 138.6 [C (Pbf)], 138.8 [2C (Ar)], 150.3, 156.1 [2CO (2Boc)], 156.5 [C (NH*C*(NHBn)=N)], 158.9 [C (Pbf)], 170.5 (C_2), 171.0 (CO_2). Minor isomer: 12.5, 18.3, 19.6 [$3CH_3$ (Pbf)], 27.2 (*CH_2*-Ind), 27.6 [C_γ (Arg)], 28.3 [$3CH_3$ (^{t}Bu)], 28.7 [$3CH_3$ (Boc)], 28.4 [C_β (Arg) and $2CH_3$ (Pbf)], 28.7 [$3CH_3$ (Boc)], 41.8 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 46.2 (C_6), 50.3 [1-CH_2 (Bn)], 51.6 [4-CH_2 and CH_2 (NHC(NH*Bn*)=N)], 52.0 (C_5-*CH*), 55.5 (C_5), 65.0 (C_3), 78.8 [C (Boc)], 81.7 [C (^{t}Bu)], 83.9 [C (Boc)], 86.9 [C (Pbf)], 115.8 [CH (Ar)], 117.5 [C (Pbf)], 118.8 [C (Ar)], 120.0, 123.1, 124.5, 124.9 [4CH (Ar)], 125.3 [C (Pbf)], 128.1, 128.8, 129.2 [10CH (Ar)], 131.9 [C (Ar)], 132.7, 135.4 [2C (Pbf)], 136.4 [C (Ar)], 138.5 [C (Pbf)], 138.8 [2C (Ar)], 150.3, 156.1 [2CO (2Boc)], 156.5 [C (NH*C*(NHBn)=N)], 158.9 [C (Pbf)], 170.4 (C_2), 171.0 (CO_2); ES-MS *m/z* 1105.3 $[M+1]^+$; Anal. calcd. for $C_{61}H_{81}N_7O_{10}S$: C, 66.34; H, 7.39; N, 8.88. Found: C, 66.58; H, 7.67; N, 8.61.

*4.6.12. (5*S*,3*S*)-1-Benzyl-5-((*S*)-1-((*tert-*butoxycarbonyl)amino-2-(1-(*tert-*butoxycarbonyl)-indol-3-yl)ethyl-4-(*tert-*butoxycarbonyl)methyl -3-(3-(3-benzyl-2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [**(S)-22a**]*

Amorphous solid (3:1 isomeric mixture, 75 mg, 20%); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 21.04 min (major isomer) and 21.82 min (minor isomer**)**; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm) Major isomer: 1.27 (s, 9H, Boc), 1.43 [s, 6H, 2CH_3 (Pbf)], 1.46 (s, 9H, ^{t}Bu), 1.65 [s, 3H, Boc (Ind)], 1.79 [m, 2H, γ-H (Arg)], 1.81 [m, 2H, β-H (Arg)], 2.01 [s, 3H, CH_3 (Pbf)], 2.43 [s, 3H, CH_3 (Pbf)], 2.51 [s, 3H, CH_3 (Pbf)], 2.70 (m, 1H, *CH_2*-Ind), 2.91 [s, 2H, CH_2 (Pbf)], 3.15 (m, 1H, *CH_2*-Ind), 3.28 (m, 1H, 5-H), 3.31 [m, 2H, δ-H (Arg)], 3.48 (m, 2H, 6-H), 3.54 [m, 1H, 3-H], 3.60 (m, 1H, 4-CH_2), 3.70 (m, 1H, 4-CH_2), 4.02 (m, 1H, 5-CH), 4.42 [d, 2H, *J* = 5.5 Hz, CH_2 (NHC(NH*Bn*)=N)], 4.58 [m, 1H, 1-CH_2 (Bn)], 4.70 [m, 1H, 1-CH_2 (Bn)], 6.14 (d, 1H, *J* = 8 Hz, *NH*Boc), 7.10-7.41 [m, 14H, Ar and *NH*C(*NH*Bn)=N], 7.50 (s, 1H, Ar), 7.59 (d, 1H, *J* = 7.5 Hz, Ar), 8.11 (d, 1H, *J* = 7.5 Hz, Ar). Minor isomer: 1.27 (s, 9H, Boc), 1.44 [s, 6H, 2CH_3 (Pbf)], 1.45 (s, 9H, ^{t}Bu), 1.65 [s, 3H, Boc (Ind)], 1.79 [m, 2H, γ-H (Arg)], 1.81 [m, 2H, β-H (Arg)], 2.01 [s, 3H, CH_3 (Pbf)], 2.43 [s, 3H, CH_3 (Pbf)], 2.51 [s, 3H, CH_3 (Pbf)], 2.70 (m, 1H, *CH_2*-Ind), 2.96 [s, 2H, CH_2 (Pbf)], 3.15 (m, 1H, *CH_2*-Ind), 3.28 (m, 1H, 5-H), 3.31 [m, 2H, δ-H (Arg)], 3.48 (m, 2H, 6-H), 3.54 [m, 1H, 3-H], 3.60 (m, 1H, 4-CH_2), 3.70 (m, 1H, 4-CH_2), 4.02 (m, 1H, 5-CH), 4.52 [m, 1H, 1-CH_2 (Bn)], 4.60 [m, 1H, CH_2 (NHC(NH*Bn*)=N)], 4.66 [m, 1H, CH_2 (NHC(NH*Bn*)=N)], 4.70 [m, 1H, 1-CH_2 (Bn)], 6.14 (d, 1H, *J* = 8 Hz, *NH*Boc), 7.10-7.41 [m, 14H, Ar and *NH*C(*NH*Bn)=N], 7.50 (s, 1H, Ar), 7.59 (d, 1H, *J* = 7.5 Hz, Ar), 8.11 (d, 1H, *J* = 7.5 Hz, Ar); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm) Major isomer: 11.8, 17.5, 18.7 [3CH_3 (Pbf)], 24.7 (*CH_2*-Ind), 25.8 [C_γ (Arg)], 27.6 [3CH_3 (^{t}Bu) and 3CH_3 (Boc)], 27.9 [2CH_3 (Pbf)], 28.0 [3CH_3 (Boc)], 31.3 [C_β (Arg)], 41.4 [C_δ (Arg)], 43.0 [CH_2 (Pbf)], 44.4 (C_6), 44.9 [CH_2 (NHC(NH*Bn*)=N)], 49.6 [1-CH_2 (Bn)], 51.5 (C_5-*CH*), 56.1 (4-CH_2), 60.4 (C_5), 64.1 (C_3), 78.3 [C (Boc)], 81.2 [C (^{t}Bu)], 83.4 [C (Boc)], 86.2 [C (Pbf)], 115.2 [CH (Ar)], 116.8 [C (Pbf)], 118.3 [C (Ar)], 119.3, 122.6, 123.6, 124.3 [4CH (Ar)], 124.6 [C (Pbf)], 127.5, 128.1, 128.4, 128.7 [10CH (Ar)], 131.1 [C (Ar)], 132.2, 135.2 [2C (Pbf)], 135.8 [C (Ar)], 137.8 [2C (Ar)], 138.0 [C (Pbf)], 149.6, 155.3 [2CO (2Boc)], 155.8 [C (NH*C*(NHBn)=N)], 158.3 [C (Pbf)], 170.4 (C_2), 170.6 (CO_2). Minor isomer: 11.8, 17.5, 18.7 [3CH_3 (Pbf)], 24.7 (*CH_2*-Ind), 25.8 [C_γ (Arg)], 27.6 [3CH_3 (^{t}Bu) and 3CH_3 (Boc)], 27.9 [2CH_3 (Pbf)], 28.0 [3CH_3 (Boc)], 31.3 [C_β (Arg)], 41.4 [C_δ (Arg)], 43.0 [CH_2 (Pbf)], 44.4 (C_6), 49.6 [1-CH_2 (Bn) and CH_2 (NHC(NH*Bn*)=N)], 51.5 (C_5-*CH*), 56.1 (4-CH_2), 60.4 (C_5), 64.1 (C_3), 78.3 [C (Boc)], 81.2 [C (^{t}Bu)], 83.4 [C (Boc)], 86.2 [C (Pbf)], 115.2 [CH (Ar)], 116.8 [C (Pbf)], 118.3 [C (Ar)], 119.3, 122.6, 123.6, 124.3 [4CH (Ar)], 124.6 [C (Pbf)], 127.5, 128.1, 128.4, 128.7 [10CH (Ar)], 131.1 [C (Ar)], 132.2, 135.2 [2C (Pbf)], 135.8 [C (Ar)], 137.8 [2C

(Ar)], 138.0 [C (Pbf)], 149.6, 155.3 [2CO (2Boc)], 155.8 [C (NH*C*(NHBn)=N)], 158.3 [C (Pbf)], 170.4 (C_2), 170.6 (CO_2); ES-MS *m/z* 1105.3 $[M+1]^+$; Anal. calcd. for $C_{61}H_{81}N_7O_{10}S$: C, 66.34; H, 7.39; N, 8.88. Found: C, 66.07; H, 7.27; N, 9.14.

*4.6.13. (5*R*,3*S*)-1-Benzyl- 5-((*S*)-1-(*tert-*butoxycarbonyl)amino-2-phenylethyl)- 4-(*tert-*butoxycarbonyl)methyl -3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino) propyl)-2-oxopiperazine [**(R)-23a**]*

Amorphous solid (152 mg, 51%); $[\alpha]_D^{20}$ -2.8 (*c* 1.0, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 6.90 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.21 (s, 9H, Boc), 1.44 [s, 6H, $2CH_3$ (Pbf)], 1.46 (s, 9H, ^{t}Bu), 1.88 [m, 4H, β-H (Arg) and γ-H (Arg)], 2.04 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.58 [s, 3H, CH_3 (Pbf)], 2.62 (dd, 1H, *J* = 11 and 14 Hz, *CH*$_2$-Ph), 2.97 [s, 2H, CH_2 (Pbf)], 3.13 (d, 1H, *J* = 16 Hz, 4-CH_2), 3.22 (m, 1H, 6-H), 3.30 [m, 2H, δ-H (Arg)], 3.35 [m, 1H, 3-H], 3.37 (m, 1H, 6-H), 3.39 (m, 1H, 5-H), 3.46 (dd, 1H, *J* = 3 and 14 Hz, *CH*$_2$-Ph), 3.60 (d, 1H, *J* = 16 Hz, 4-CH_2), 3.82 (dt, 1H, *J* = 3.5 and 12 Hz, 5-CH), 4.35 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 4.83 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 5.89 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 6.55 [m, 3H, $NHC(NH_2)$=N], 7.10-7.38 (m, 10H, Ar); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 12.7, 18.2, 19.5 [$3CH_3$ (Pbf)], 27.4 [C_γ (Arg)], 28.3 [$3CH_3$ (^{t}Bu)], 28.5 [$2CH_3$ (Pbf)], 28.7 [$3CH_3$ (Boc)], 28.9 [C_β (Arg)], 38.0 (*CH*$_2$-Ph), 41.6 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 46.0 (C_6), 50.2 [1-CH_2 (Bn)], 51.7 (4-CH_2), 53.6 (C_5-*CH*), 55.7 (C_5), 65.3 (C_3), 78.8 [C (Boc)], 81.5 [C (^{t}Bu)], 86.9, 117.4, 125.3 [3C (Pbf)], 126.7, 128.1, 128.7, 128.8, 129.4, 130.2 [10CH (Ar)], 132.8, 135.6, 138.7 [3C (Pbf)], 138.6, 140.4 [C (Ar)], 156.3 [CO (Boc)], 157.4 [C ($NHC(NH_2)$=N)], 158.9 [C (Pbf)], 170.6 (C_2), 170.9 (CO_2); ES-MS *m/z* 876.1 $[M+1]^+$; Anal. calcd. for $C_{47}H_{66}N_6O_8S$: C, 64.51; H, 7.60; N, 9.60. Found: C, 64.25; H, 7.38; N, 9.94.

*4.6.14. (5*S*,3*S*)-1-Benzyl- 5-((*S*)-1-(*tert-*butoxycarbonyl)amino-2-phenylethyl)- 4-(*tert-*butoxycarbonyl)methyl -3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino) propyl)-2-oxopiperazine [**(S)-23a**]*

Amorphous solid (149 mg, 50%); $[\alpha]_D^{20}$ +16 (*c* 1.0, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 6.80 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.25 (s, 9H, Boc), 1.43 [s, 6H, $2CH_3$ (Pbf)], 1.46 (s, 9H, ^{t}Bu), 1.75 [m, 4H, β-H (Arg) and γ-H (Arg)], 2.05 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.57 [s, 3H, CH_3 (Pbf)], 2.55 (m, 1H, *CH*$_2$-Ph), 2.98 [s, 2H, CH_2 (Pbf)], 3.03 (d, 1H, *J* = 13 Hz, *CH*$_2$-

Ph), 3.22 (m, 1H, 5-H), 3.24 [m, 2H, δ-H (Arg)], 3.40 (m, 2H, 6-H), 3.46 (m, 1H, 3-H), 3.53 (d, 1H, *J* = 18 Hz, 4-CH_2), 3.67 (d, 1H, *J* = 18 Hz, 4-CH_2), 3.82 (m, 1H, 5-CH), 4.53 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 4.70 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 6.10 (d, 1H, *J* = 8 Hz, *NH*Boc), 6.50 [m, 3H, NHC(NH_2)=N], 7.10-7.40 (m, 10H, Ar); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) *δ* (ppm): 12.6, 18.2, 19.5 [3CH_3 (Pbf)], 26.4 [C_γ (Arg)], 28.3 [3CH_3 (^{t}Bu)], 28.5 [2CH_3 (Pbf)], 28.7 [3CH_3 (Boc)], 32.2 [C_β (Arg)], 35.5 (*CH_2*-Ph), 41.6 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 44.9 (C_6), 49.9 [1-CH_2 (Bn)], 54.0 (C_5-*CH*), 56.4 (4-CH_2), 60.7 (C_5), 65.0 (C_3), 78.8 [C (Boc)], 81.7 [C (^{t}Bu)], 86.9, 117.4, 125.3 [3C (Pbf)], 126.8, 128.1, 128.8, 129.0, 129.4, 129.9 [10CH (Ar)], 132.8, 135.7, 138.7 [3C (Pbf)], 138.5, 140.6 [C (Ar)], 156.1 [CO (Boc)], 157.3 [C (NHC(NH_2)=N)], 158.9 [C (Pbf)], 171.1 (C_2), 171.3 (CO_2); ES-MS *m/z* 876.1 $[M+1]^+$; Anal. calcd. for $C_{47}H_{66}N_6O_8S$: C, 64.51; H, 7.60; N, 9.60. Found: C, 64.68; H, 7.42; N, 9.72.

*4.6.15. (5*R,*3*S*)-1-Benzyl- 5-((*S*)-1-(*tert-*butoxycarbonyl)amino-2-phenylethyl)- 4-(*tert-*butoxycarbonyl)methyl -3-(3-(3-benzyl-2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino) propyl)-2-oxopiperazine [(**R**)-**25a**]*

Amorphous solid (5:1 isomeric mixture, 46 mg, 14%); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 12.82 min (major isomer) and 12.53 min (minor isomer); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) *δ* (ppm) Major isomer: 1.20 (s, 9H, Boc), 1.44 [s, 6H, 2CH_3 (Pbf)], 1.45 (s, 9H, ^{t}Bu), 1.85 [m, 4H, β-H (Arg) and γ-H (Arg)], 2.01 [s, 3H, CH_3 (Pbf)], 2.44 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.64 (dd, 1H, *J* = 11 and 14 Hz, *CH_2*-Ph), 2.93 [s, 2H, CH_2 (Pbf)], 3.14 (d, 1H, *J* = 16 Hz, 4-CH_2), 3.26 (m, 1H, 6-H), 3.36 [m, 2H, δ-H (Arg) and 3-H], 3.40 (m, 1H, 5-H), 3.42 (m, 1H, 6-H), 3.47 [m, 1H, δ-H (Arg)], 3.48 (dd, 1H, *J* = 3 and 14 Hz, *CH_2*-Ph), 3.62 (d, 1H, *J* = 16 Hz, 4-CH_2), 3.82 (m, 1H, 5-CH), 4.36 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 4.43 [dd, 1H, *J* = 6 and 16 Hz, CH_2 (NHC(NH*Bn*)=N)], 4.46 [dd, 1H, *J* = 6 and 16 Hz, CH_2 (NHC(NH*Bn*)=N)], 4.83 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 5.86 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 7.09-7.39 (m, 17H, Ar and *NH*C(*NH*Bn)=N). Minor isomer: 1.22 (s, 9H, Boc), 1.43 [s, 6H, 2CH_3 (Pbf)], 1.45 (s, 9H, ^{t}Bu), 1.85 [m, 4H, β-H (Arg) and γ-H (Arg)], 2.01 [s, 3H, CH_3 (Pbf)], 2.45 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.58 (m, 1H, *CH_2*-Ph), 2.95 [s, 2H, CH_2 (Pbf)], 3.16 (d, 1H, *J* = 16 Hz, 4-CH_2), 3.26 (m, 1H, 6-H), 3.36 [m, 2H, δ-H (Arg) and 3-H], 3.42 (m, 1H, 6-H), 3.47 [m, 1H, δ-H (Arg)], 3.46 (m, 1H, *CH_2*-Ph), 3.63 (d, 1H, *J* = 16 Hz, 4-CH_2), 3.82 (m, 1H, 5-CH), 4.36 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 4.67 [d, 1H, *J* = 16 Hz, CH_2 (NHC(NH*Bn*)=N)], 4.75

[d, 1H, *J* = 16 Hz, CH_2 (NHC(NH*Bn*)=N)], 4.85 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 5.85 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 7.09-7.39 (m, 17H, Ar and *NH*C(*NH*Bn)=N); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm) Major isomer: 11.9, 17.6, 18.9 [$3CH_3$ (Pbf)], 26.6 [C_γ (Arg)], 27.6 [$3CH_3$ (^{t}Bu)], 27.8 [$3CH_3$ (Boc)], 28.0 [$2CH_3$ (Pbf) and C_β (Arg)], 37.4 (*CH_2*-Ph), 41.2 [C_δ (Arg)], 42.9 [CH_2 (Pbf)], 44.7 [CH_2 (NHC(NH*Bn*)=N)], 45.4 (C_6), 49.6 [1-CH_2 (Bn)], 50.9 (4-CH_2), 52.6 (C_5-*CH*), 55.0 (C_5), 64.4 (C_3), 78.1 [C (Boc)], 80.9 [C (^{t}Bu)], 86.3, 116.8, 124.6 [3C (Pbf)], 126.1, 127.5, 128.6, 128.2, 128.8, 129.6 [15CH (Ar)], 132.1, 134.8 [2C (Pbf)], 137.9 [2C (Ar)], 138.0 [C (Pbf)], 139.7 [C (Ar)], 153.9 [C (NH*C*(NHBn)=N)], 155.9 [CO (Boc)], 158.3 [C (Pbf)], 170.1 (C_2), 170.4 (CO_2). Minor isomer: 11.9, 17.6, 18.9 [$3CH_3$ (Pbf)], 26.6 [C_γ (Arg)], 27.6 [$3CH_3$ (^{t}Bu)], 27.8 [$3CH_3$ (Boc)], 28.0 [$2CH_3$ (Pbf) and C_β (Arg)], 37.4 (*CH_2*-Ph), 41.2 [C_δ (Arg)], 42.9 [CH_2 (Pbf)], 45.4 (C_6), 49.6 [1-CH_2 (Bn)], 50.7 (4-CH_2), 50.9 [CH_2 (NHC(NH*Bn*)=N)], 52.6 (C_5-*CH*), 64.4 (C_3), 78.1 [C (Boc)], 80.9 [C (^{t}Bu)], 86.3, 116.8, 124.6 [3C (Pbf)], 126.1, 127.5, 127.9, 128.2, 128.5, 129.5 [15CH (Ar)], 132.0, 134.8 [2C (Pbf)], 137.9 [2C (Ar)], 138.0 [C (Pbf)], 139.7 [C (Ar)], 153.9 [C (NH*C*(NHBn)=N)], 155.9 [CO (Boc)], 158.2 [C (Pbf)], 169.9 (C_2), 170.4 (CO_2); ES-MS *m/z* 966.2 $[M+1]^+$; Anal. calcd. for $C_{54}H_{72}N_6O_8S$: C, 67.19; H, 7.52; N, 8.71. Found: C, 67.45; H, 7.38; N, 9.02.

*4.6.16. (5*S,*3*S*)-1-Benzyl- 5-((*S*)-1-(*tert-*butoxycarbonyl)amino-2-phenylethyl)- 4-(*tert-*butoxycarbonyl)methyl -3-(3-(3-benzyl-2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino) propyl)-2-oxopiperazine [**(S)-25a**]*

Amorphous solid (3:1 isomeric mixture, 49 mg, 15%); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 12.28 min (major isomer) and 12.83 min (minor isomer); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm) Major isomer: 1.25 (s, 9H, Boc), 1.45 [s, 6H, $2CH_3$ (Pbf)], 1.46 (s, 9H, ^{t}Bu), 1.77 [m, 4H, β-H (Arg) and γ-H (Arg)], 2.02 [s, 3H, CH_3 (Pbf)], 2.44 [s, 3H, CH_3 (Pbf)], 2.52 [s, 3H, CH_3 (Pbf)], 2.54 (m, 1H, *CH_2*-Ph), 2.95 [s, 2H, CH_2 (Pbf)], 3.03 (m, 1H, *CH_2*-Ph), 3.24 (m, 1H, 5-H), 3.30 [m, 2H, δ-H (Arg)], 3.40 (m, 2H, 6-H), 3.51 (m, 1H, 3-H), 3.54 (m, 1H, 4-CH_2), 3.68 (m, 1H, 4-CH_2), 3.88 (m, 1H, 5-CH), 4.44 [d, 1H, *J* = 5.5 Hz, CH_2 (NHC(NH*Bn*)=N)], 4.52 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 4.70 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 6.08 (d, 1H, *J* = 8.5 Hz, *NH*Boc), 7.10-7.37 (m, 17H, Ar and *NH*C(*NH*Bn)=N). Minor isomer: 1.24 (s, 9H, Boc), 1.44 [s, 6H, $2CH_3$ (Pbf)], 1.45 (s, 9H, ^{t}Bu), 1.77 [m, 4H, β-H (Arg) and γ-H (Arg)], 2.02 [s, 3H, CH_3 (Pbf)], 2.47 [s, 3H, CH_3 (Pbf)], 2.52 [s, 3H, CH_3 (Pbf)], 2.54 (m, 1H, *CH_2*-Ph), 2.97 [s, 2H, CH_2 (Pbf)], 3.03 (m, 1H, *CH_2*-Ph), 3.24 (m, 1H, 5-H), 3.30 [m, 2H, δ-H (Arg)], 3.40 (m, 2H, 6-H), 3.51 (m, 1H, 3-

H), 3.59 (m, 1H, 4-CH_2), 3.63 (m, 1H, 4-CH_2), 3.88 (m, 1H, 5-CH), 4.48 [m, 1H, CH_2 (NHC(NH*Bn*)=N)], 4.58 [m, 1H, 1-CH_2 (Bn)], 4.64 [m, 1H, 1-CH_2 (Bn)], 6.08 (d, 1H, *J* = 8.5 Hz, *NH*Boc), 7.10-7.37 [m, 17H, Ar and *NH*C(*NH*Bn)=N]; ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm) Major isomer: 12.6, 18.2, 19.5 [3CH_3 (Pbf)], 26.3 [C_γ (Arg)], 28.4 [3CH_3 (Boc)], 28.5 [2CH_3 (Pbf)], 28.8 [3CH_3 (^{t}Bu)], 32.1 [C_β (Arg)], 35.3 (*CH_2*-Ph), 42.0 [C_δ (Arg)], 43.7 [CH_2 (Pbf)], 44.9 [C_6 and CH_2 (NHC(NH*Bn*)=N)], 50.0 [1-CH_2 (Bn)], 53.9 (C_5-*CH*), 56.2 (4-CH_2), 60.7 (C_5), 64.8 (C_3), 78.1 [C (Boc)], 81.7 [C (^{t}Bu)], 86.9, 117.5, 125.3 [3C (Pbf)], 126.8, 128.1, 128.7, 129.0, 129.4, 129.9 [15CH (Ar)], 132.8, 135.6 [2C (Pbf)], 138.4 [2C (Ar)], 138.7 [C (Pbf)], 140.7 [C (Ar)], 156.0 [CO (Boc) and C (NH*C*(NHBn)=N)], 158.9 [C (Pbf)], 171.1 (C_2), 171.2 (CO_2). Minor isomer: 12.6, 18.2, 19.5 [3CH_3 (Pbf)], 26.3 [C_γ (Arg)], 28.4 [3CH_3 (Boc)], 28.5 [2CH_3 (Pbf)], 28.8 [3CH_3 (^{t}Bu)], 32.1 [C_β (Arg)], 35.3 (*CH_2*-Ph), 42.0 [C_δ (Arg)], 43.7 [CH_2 (Pbf)], 44.9 (C_6), 50.0 [1-CH_2 (Bn)], 52.6 [CH_2 (NHC(NH*Bn*)=N)], 53.9 (C_5-*CH*), 56.2 (4-CH_2), 60.7 (C_5), 64.8 (C_3), 78.7 [C (Boc)], 81.7 [C (^{t}Bu)], 86.9, 117.5, 125.3 [3C (Pbf)], 126.8, 128.1, 128.9, 129.2, 129.4, 129.9 [15CH (Ar)], 132.7, 135.6 [2C (Pbf)], 138.4 [2C (Ar)], 138.7 [C (Pbf)], 140.7 [C (Ar)], 156.0 [CO (Boc)], 156.1 [C (NH*C*(NHBn)=N)], 158.9 [C (Pbf)], 171.1 (C_2), 171.2 (CO_2); ES-MS *m/z* 966.2 $[M+1]^+$; Anal. Calcd. for $C_{54}H_{72}N_6O_8S$: C, 67.19; H, 7.52; N, 8.71. Found: C, 67.37; H, 7.74; N, 8.84.

*4.7. General procedure for the benzylation of the 4-alkyl-2-oxopiperazines **10b** and **12b**. Synthesis of the 4-alkyl-1-benzyl-2-oxopiperazines **(R)-** and **(S)-(26b** and **27b)***

NaH (60% suspension in mineral oil, 9 mg, 0.38 mmol) and benzyl bromide (0.45 µL, 0.38 mmol) were added to a solution of the corresponding 2-oxopiperazine **10b** and **12b** (0.34 mmol) in anhydrous mixture THF/DMF (9:1, 10 mL) under argon at 0 ºC. After 1h of stirring, the crude reaction mixture was diluted with EtOAc (100 mL) and the excess of NaH was hydrolysed by addition of H_2O (20 mL). The aqueous layer was extracted with EtOAc (2×50 mL) and the organic extracts were dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography, using MeOH in CH_2Cl_2 gradient as mobile phase to obtain the respective 4-alkyl-1-benzyl-2-oxopiperazine derivatives **(*R*)-** and **(*S*)-**(**26b** and **27b**) in 70-80 %. The compounds were dissolved in CH_3CN/H_2O (1:2, 2 mL) and the solutions were lyophilized.

*4.7.1. (5*R,*3*S*)-1,4-Dibenzyl-5-((*S*)-1-((*tert-*butoxycarbonyl)amino-2-(1-(*tert-*butoxy-carbonyl)-indol-3-yl)ethyl-3-(4-((*tert-*butoxycarbonyl)amino)butyl)-2-oxopiperazine [**(R)-26b**]*

Amorphous solid (220 mg, 80%); $[\alpha]_D^{20}$ -15 (*c* 1.4, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 12.75 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.25 (s, 9H, Boc), 1.31 [m, 4H, γ-H (Lys) and δ-H (Lys)], 1.36 [s, 9H, Boc (Lys)], 1.61 [m, 1H, β-H (Lys)], 1.64 [s, 9H, Boc (Ind)], 1.80 [m, 1H, β-H (Lys)], 1.86 [m, 1H, ε-H (Lys)], 2.80 (m, 1H, *CH*$_2$-Ind), 3.03 [m, 1H, ε-H (Lys)], 3.17 (dd, 1H, *J* = 4.5 and 10 Hz, 3-H), 3.37 (m, 1H, 6-H), 3.54 [m, 1H, 4-CH_2 (Bn)], 3.59 (m, 3H, 5-H, 6-H and *CH*$_2$-Ind), 4.17 (m, 1H, 5-CH), 4.18 [d, 1H, *J* = 13.5 Hz, 4-CH_2 (Bn)], 4.51 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 4.79 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 5.84 [m, 1H, *NH*Boc (Lys)], 6.00 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 7.16 (t, 1H, *J* = 7.5 Hz, Ar), 7.22-7.57 (m, 13H, Ar), 8.12 (d, 1H, *J* = 7.5 Hz, Ar); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 24.5 [C_γ (Lys)], 27.9 (*CH*$_2$-Ind), 28.3, 28.5, 28.7 [9CH_3 (3Boc)], 30.3 [C_δ (Lys)], 31.2 [C_β (Lys)], 41.1 [C_ε (Lys)], 45.8 (C_6), 50.2 [1-CH_2 (Bn)], 51.3 (C_5-*CH*), 51.9 [4-CH_2 (Bn)], 55.6 (C_5), 62.5 (C_3), 78.2, 78.9, 83.9 [3C (3Boc)], 115.8 [CH (Ar)], 118.7 [C (Ar)], 119.9, 123.2, 124.3, 124.9, 128.1, 128.2, 128.9, 129.3, 129.4, 129.9 [14CH (Ar)], 131.9, 136.3, 138.9, 139.5 [4C (Ar)], 150.3, 156.5 [3CO (3Boc)], 171.0 (C_2); ES-MS *m/z* 811.2 $[M+1]^+$; Anal. calcd. for $C_{47}H_{63}N_5O_7$: C, 69.69; H, 7.84; N, 8.65. Found: C, 69.92; H, 8.06; N, 8.41.

*4.7.2. (5*S,*3*S*)-1,4-Dibenzyl-5-((*S*)-1-((*tert-*butoxycarbonyl)amino-2-(1-(*tert-*butoxy-carbonyl)-indol-3-yl)ethyl-3-(4-((*tert-*butoxycarbonyl)amino)butyl)-2-oxopiperazine [**(S)-26b**]*

Amorphous solid (220 mg, 80%); $[\alpha]_D^{20}$ +11 (*c* 1.3, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 12.99 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.28 [m, 2H, γ-H (Lys)], 1.31 [m, 9H, Boc], 1.34 [m, 2H, δ-H (Lys)], 1.37 [m, 9H, Boc (Lys)], 1.43 [m, 1H, β-H (Lys)], 1.66 [s, 9H, Boc (Ind)], 1.71 [m, 1H, β-H (Lys)], 2.85 (m, 1H, *CH*$_2$-Ind), 2.95 [m, 2H, ε-H (Lys)], 3.16 (m, 1H, 5-H), 3.19 (m, 1H, *CH*$_2$-Ind), 3.27 (t, 1H, *J* = 7 Hz, 3-H), 3.46 (dd, 1H, *J* = 5 and 13.5 Hz, 6-H), 3.57 (m, 1H, 6-H), 3.85 [d, 1H, *J* = 14 Hz, 4-CH_2 (Bn)], 4.08 [d, 1H, *J* = 14 Hz, 4-CH_2 (Bn)], 4.12 (m, 1H, 5-CH), 4.57 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 4.66 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 5.80 [m, 1H, *NH*Boc (Lys)], 6.09 (d, 1H, *J* = 9 Hz, *NH*Boc), 7.18-7.38 (m, 10H, Ar), 7.47 (m, 3H, Ar),

7.61 (d, 1H, *J* = 7.5 Hz, Ar), 8.12 (d, 1H, *J* = 7.5 Hz, Ar). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 24.2 [C_γ (Lys)], 26.4 (*CH_2*-Ind), 28.3, 28.7 [9CH_3 (3Boc)], 31.2 [C_δ (Lys)], 34.8 [C_β (Lys)], 41.0 [C_ε (Lys)], 45.0 [C_6], 49.8 [1-CH_2 (Bn)], 52.8 (C_5-*CH*), 62.3 [4-CH_2 (Bn)], 63.6 (C_5), 64.3 (C_3), 78.2, 78.8, 84.0 [3C (3Boc)], 115.9 [CH (Ar)], 119.0 [C (Ar)], 119.9, 123.2, 124.0, 125.0, 128.1, 128.7, 129.1, 129.4, 130.2 [14CH (Ar)], 131.8, 136.3, 138.7, 139.9 [4C (Ar)], 150.3, 156.5 [3CO (3Boc)], 171.7 (C_2); ES-MS *m/z* 811.2 $[M+1]^+$; Anal. calcd. for $C_{47}H_{63}N_5O_7$: C, 69.69; H, 7.84; N, 8.65. Found: C, 69.90; H, 7.96; N, 8.52.

*4.7.3. (5*R,*3*S*)-1-Benzyl-5-((*S*)-1-((*tert-*butoxycarbonyl)amino-2-(1-(*tert-*butoxycarbonyl)-indol-3-yl)ethyl-4-(*tert-*butoxycarbonyl)methyl -3-(4-((*tert-*butoxycarbonyl)amino)-butyl)-2-oxopiperazine [(**R**)-27b]*

Amorphous solid (227 mg, 80%); $[\alpha]_D^{20}$ -4.2 (*c* 1.5, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 11.80 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.23 (s, 9H, Boc), 1.37 [s, 9H, Boc (Lys)], 1.48 (s, 9H, ^{t}Bu), 1.61 [m, 2H, γ-H (Lys)], 1.63 [m, 2H, δ-H (Lys)], 1.65 [s, 9H, Boc (Ind)], 1.79 [m, 1H, β-H (Lys)], 1.87 [m, 1H, β-H (Lys)], 2.75 (dd, 1H, *J* = 10.5 and 15 Hz, *CH_2*-Ind), 3.13 (m, 1H, 4-CH_2), 3.16 [m, 2H, ε-H (Lys)], 3.29 (m, 1H, 6-H), 3.33 (m, 1H, 3-H), 3.40 (m, 1H, 6-H), 3.46 (m, 1H, 5-H), 3.61 (m, 1H, *CH_2*-Ind), 3.62 (d, 1H, *J* = 16 Hz, 4-CH_2), 3.91 (m, 1H, 5-CH), 4.38 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 4.84 [d, 1H, *J* = 14.5 Hz, 1-CH_2 (Bn)], 5.93 [m, 1H, *NH*Boc (Lys)], 5.98 (d, 1H, *J* = 9.5 Hz, *NH*Boc), 7.22 (t, 1H, *J* = 7.5 Hz, Ar), 7.25-7.37 (m, 6H, Ar), 7.47 (s, 1H, Ind), 7.62 (d, 1H, *J* = 7.5 Hz, Ind), 8.11 (d, 1H, *J* = 7.5 Hz, Ind); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 24.7 [C_γ (Lys)], 27.6 (*CH_2*-Ind), 28.3 [6CH_3 (Boc and ^{t}Bu)], 28.5, 28.7 [6CH_3 (2Boc)], 30.4 [C_δ (Lys)], 31.3 [C_β (Lys)], 41.2 [C_ε (Lys)], 46.0 (C_6), 50.2 [1-CH_2 (Bn)], 51.9 (C_5-*CH*), 52.1 (4-CH_2), 56.0 (C_5), 65.6 (C_3), 78.2, 78.9 [2C (2Boc)], 81.5 [C (^{t}Bu)], 83.9 [C (Boc)], 115.8 [CH (Ar)], 118.9 [C (Ar)], 120.0, 123.1, 124.4, 124.9, 128.1, 128.8, 129.4 [9CH (Ar)], 131.9, 136.3, 138.7 [3C (Ar)], 150.3, 156.4 [3CO (3Boc)], 170.7 [CO_2], 170.8 (C_2); ES-MS *m/z* 835.1 $[M+1]^+$; Anal. calcd. for $C_{46}H_{67}N_5O_9$: C, 66.24; H, 8.10; N, 8.40. Found: C, 66.03; H, 7.86; N, 8.35.

*4.7.4. (5*S,*3*S*)-1-Benzyl-5-((*S*)-1-((*tert-*butoxycarbonyl)amino-2-(1-(*tert-*butoxycarbonyl)-indol-3-yl)ethyl-4-(*tert-*butoxycarbonyl)methyl -3-(4-((*tert-*butoxycarbonyl)amino)-butyl)-2-oxopiperazine [(**S**)-27b]*

Amorphous solid (198 mg, 70%); $[\alpha]_D^{20}$ +7.0 (*c* 1.3, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 80-100% gradient of solvent A in B, 30 min] t_R 12.41 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.29 (s, 9H, Boc), 1.37 [s, 9H, Boc (Lys)], 1.45 (s, 9H, ^{t}Bu), 1.55 [m, 2H, γ-H (Lys)], 1.57 [m, 2H, δ-H (Lys)], 1.65 [s, 9H, Boc (Ind)], 1.80 [m, 2H, β-H (Lys)], 2.71 (dd, 1H, J = 11 and 15 Hz, *CH*$_2$-Ind), 3.17 (dd, 1H, J = 2 and 15 Hz, *CH*$_2$-Ind), 3.09 [m, 2H, ε-H (Lys)], 3.30 (m, 1H, 5-H), 3.44 (m, 1H, 3-H), 3.47 (m, 2H, 6-H), 3.58 (d, 1H, *J* = 18 Hz, 4-CH_2), 3.70 (d, 1H, *J* = 18 Hz, 4-CH_2), 4.02 (m, 1H, 5-CH), 4.64 [m, 2H, 1-CH_2 (Bn)], 5.92 [m, 1H, *NH*Boc (Lys)], 6.14 (d, 1H, *J* = 8.5 Hz, *NH*Boc), 7.18-7.41 (m, 7H, Ar), 7.48 (s, 1H, Ar), 7.61 (d, 1H, *J* = 7.5 Hz, Ar), 8.11 (d, 1H, *J* = 7.5 Hz, Ar). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 23.9 [C_γ (Lys)], 25.3 (*CH*$_2$-Ind), 28.3 [6CH_3 (Boc and ^{t}Bu)], 28.6, 28.7 [6CH_3 (2Boc)], 30.8 [C_δ (Lys)], 35.0 [C_β (Lys)], 41.1 [C_ε (Lys)], 44.8 [C_6], 50.0 [1-CH_2 (Bn)], 51.9 (C_5-*CH*), 56.9 (4-CH_2), 60.9 (C_5), 65.6 (C_3), 78.2, 78.8 [2C (2Boc)], 81.7 [C (^{t}Bu)], 84.0 [C (Boc)], 115.9 [CH (Ar)], 119.1 [C (Ar)], 119.9, 123.3, 124.1, 125.0, 128.1, 128.8, 129.4 [9CH (Ar)], 131.7, 136.4, 138.7 [3C (Ar)], 150.3, 156.6 [3CO (3Boc)], 171.2 (CO_2), 171.3 (C_2); ES-MS *m/z* 835.1 $[M+1]^+$; Anal. calcd. for $C_{46}H_{67}N_5O_9$: C, 66.24; H, 8.10; N, 8.40. Found: C, 65.98; H, 8.36; N, 8.61.

4.8. General procedure for the synthesis of the 1,4-dibenzyl-2-oxopiperazine-derived benzyl ureas ***(R)-*** *and* ***(S)-(28a*** *and* ***29a)***

The corresponding 1,4-dibenzyl-2-oxopiperazine **14a** and **17a** (0.10 mmol) was dissolved in 3.4 N solution of HCl in EtOAc (5 mL) and the mixture was stirred at room temperature for 30 min. Afterward, the solvent was evaporated to dryness and the residue was dissolved in CH_3CN/H_2O (1:3, 2 mL) and the solution was lyophilized. The residue was dissolved in dry THF (10 mL) and TEA (56 μL, 0.40 mmol) and benzyl isocyanate (15 μL, 0.12 mmol) were successively added to the solution. After 1 h of stirring at room temperature, the solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (40 mL). The solution was successively washed with H_2O (10 mL) and brine (10 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by flash chromatography, using a MeOH in EtOAc gradient as mobile phase to obtain the resèctove 1,4-dibenzyl-2-oxopiperazine-derived benzyl ureas **28a** and **29a** in 50-85 % yield. The purified compounds were dissolved in CH_3CN/H_2O (1:2, 2 mL) and the solutions were lyophilized.

*4.8.1. (5*R*,3*S*)-1,4-Dibenzyl-5-((*S*)-1-(3-benzylureido)-2-(1*H*-indol-3-yl)ethyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [**(R)-28a**]*

Amorphous solid (46 mg, 50%); $[\alpha]_D^{20}$ -13 (*c* 1.2, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 50-100% gradient of solvent A in B, 30 min] t_R 11.23 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.42 [s, 6H, $2CH_3$ (Pbf)], 1.79 [m, 4H, β-H and γ-H (Arg)], 2.02 [s, 3H, CH_3 (Pbf)], 2.49 [s, 3H, CH_3 (Pbf)], 2.58 [s, 3H, CH_3 (Pbf)], 2.92 [s, 2H, CH_2 (Pbf)], 2.98 (m, 1H, *CH*$_2$-Ind), 3.06 [m, 2H, δ-H (Arg)], 3.11 [m, 1H, 3-H], 3.39 (dd, 1H, *J* = 4.5 and 12.5 Hz, 6-H), 3.49 [m, 1H, 4-CH_2 (Bn)], 3.53 [m, 2H, *CH*$_2$-Ind and 5-H], 3.65 (t, 1H, *J* = 12.5 Hz, 6-H), 4.20 [m, 2H, CH_2 (Bn, Urea)], 4.23 [m, 1H, 4-CH_2 (Bn)], 4.40 [m, 1H, 5-CH], 4.51 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 4.69 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 5.55 (d, 1H, *J* = 9.5 Hz, 5-CH-*NH*), 5.79 (m, 1H, CO*NH*), 6.52 [br s, 3H, NHC(NH_2)=N], 6.91 (t, 1H, *J* = 7.5 Hz, Ar), 7.05 (t, 1H, *J* = 7.5 Hz, Ar), 7.11 (d, 1H, *J* = 7.5 Hz, Ar), 7.14-7.39 (m, 15H, Ar), 7.41 (d, 1H, *J* = 7.5 Hz, Ar), 7.49 (d, 1H, *J* = 7.5 Hz, Ar), 10.08 [s, 1H, NH (Ar)]; ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 12.6, 18.3, 19.5 [$3CH_3$ (Pbf)], 27.2 (*CH*$_2$-Ind), 28.1 [C_γ (Arg)], 28.7 [$2CH_3$ (Pbf)], 28.8 [C_β (Arg)], 41.2 [C_δ (Arg)], 43.5 [CH_2 (Pbf)], 44.1 [CH_2 (Bn, Urea)], 46.2 (C_6), 50.4 [1-CH_2 (Bn)], 51.4 (C_5-*CH*), 51.9 [4-CH_2 (Bn)], 55.5 (C_5), 62.2 (C_3), 87.0 [C (Pbf)], 112.1 [C (Ar)], 112.5 [CH (Ar)], 117.5 [C (Pbf)], 119.4, 121.9, 124.1 [4CH (Ar)], 125.3 [C (Pbf)], 127.3, 127.7, 128.1, 128.6, 129.3, 129.4, 129.9 [15CH (Ar)], 129.0 [C (Ar)], 132.8, 135.4 [2C (Pbf)], 137.5 [C (Ar)], 138.7 [C (Pbf)], 138.8, 139.6, 141.8 [C (Ar)], 157.4 [C (NHC(NH_2)=N)], 158.8 [C (Pbf)], 158.9 [CO (Urea)], 171.0 (C_2); ES-MS *m/z* 924.1 $[M+1]^+$; Anal. calcd. for $C_{53}H_{62}N_8O_5S$: C, 68.95; H, 6.77; N, 12.14. Found: C, 68.61; H, 6.89; N, 12.06.

*4.8.2. (5*S*,3*S*)-1,4-Dibenzyl-5-((*S*)-1-(3-benzylureido)-2-(1*H*-indol-3-yl)ethyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [**(S)-28a**]*

Amorphous solid (46 mg, 50%); $[\alpha]_D^{20}$ +11 (*c* 1.1, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 50-100% gradient of solvent A in B, 30 min] t_R 13.75 min; ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ (ppm): 1.30 [m, 1H, γ-H (Arg)], 1.43 [s, 6H, $2CH_3$ (Pbf)], 1.54 [m, 2H, β-H and γ-H (Arg)], 1.73 [m, 1H, β-H (Arg)], 2.04 [s, 3H, CH_3 (Pbf)], 2.49 [s, 3H, CH_3 (Pbf)], 2.57 [s, 3H, CH_3 (Pbf)], 2.77 (m, 1H, *CH*$_2$-Ind), 2.88 [m, 1H, δ-H (Arg)], 2.96 [s, 2H, CH_2 (Pbf)],

3.09 [m, 1H, δ-H (Arg)], 3.10 [m, 1H, 3-H], 3.12 (m, 2H, 5-H and *CH*$_2$-Ind), 3.47 (m, 2H, 6-H), 3.74 [d, 1H, *J* = 13.5 Hz, 4-CH$_2$ (Bn)], 3.96 [d, 1H, *J* = 13.5 Hz, 4-CH$_2$ (Bn)], 4.24 [m, 1H, 5-CH], 4.26 [dd, 1H, *J* = 5.5 and 15 Hz, CH$_2$ (Bn, Urea)], 4.30 [dd, 1H, *J* = 6 and 15 Hz, CH$_2$ (Bn, Urea)], 4.46 [d, 1H, *J* = 15 Hz, 1-CH$_2$ (Bn)], 4.71 [d, 1H, *J* = 15 Hz, 1-CH$_2$ (Bn)], 5.70 (d, 1H, *J* = 8.5 Hz, 5-CH-*NH*), 6.03 (m, 1H, CO*NH*), 6.45 [br s, 3H, NHC(NH$_2$)=N], 6.96 (t, 1H, *J* = 7.5 Hz, Ar), 7.02 (m, 1H, Ar), 7.08 (t, 1H, *J* = 7.5 Hz, Ar), 7.15-7.39 (m, 15H, Ar), 7.40 (d, 1H, *J* = 7.5 Hz, Ar), 7.53 (d, 1H, *J* = 7.5 Hz, Ar), 10.00 [s, 1H, NH (Ar)]; ^{13}C NMR (125 MHz, (CD$_3$)$_2$CO) *δ* (ppm): 12.6, 18.3, 19.5 [3CH$_3$ (Pbf)], 27.0 [C$_\gamma$ (Arg)], 28.3 (*CH*$_2$-Ind), 28.7 [2CH$_3$ (Pbf)], 31.1 [C$_\beta$ (Arg)], 40.9 [C$_\delta$ (Arg)], 43.6 [CH$_2$ (Pbf)], 44.3 [CH$_2$ (Bn, Urea)], 45.9 (C$_6$), 49.7 [1-CH$_2$ (Bn)], 54.1 (C$_5$-*CH*), 62.9 [C$_5$ and 4-CH$_2$ (Bn)], 63.6 (C$_3$), 86.9 [C (Pbf)], 112.1 [C and CH (Ar)], 117.4 [C (Pbf)], 119.4, 122.0, 123.7 [4CH (Ar)], 125.4 [C (Pbf)], 127.4, 127.9, 128.1, 128.2, 128.8, 129.5, 130.3 [15CH (Ar)], 129.1 [C (Ar)], 132.8, 135.7 [2C (Pbf)], 137.5 [C (Ar)], 138.7 [C (Pbf) and C (Ar)], 140.0, 141.7 [2C (Ar)], 157.5 [C (NHC(NH$_2$)=N)], 158.9 [C (Pbf)], 159.2 [CO (Urea)], 172.1 (C$_2$); ES-MS *m/z* 924.2 [M+1]$^+$; Anal. calcd. for $C_{53}H_{62}N_8O_5S$: C: 68.95, H: 6.77, N: 12.14. Found: C: 68.59, H: 6.98, N: 12.02.

*4.8.3. (5*R,*3*S*)-1,4-Dibenzyl-5-((*S*)-1-(3-benzylureido)-2-phenylethyl)-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino) propyl)-2-oxopiperazine [**(R)-29a**]*

Amorphous solid (62 mg, 70%); $[\alpha]_D^{20}$ -14 (*c* 1.3, CH_2Cl_2); HPLC [Sunfire C$_{18}$ (4.6 × 150 mm, 3.5 μm), 60-100% gradient of solvent A in B, 30 min] t_R 8.12 min; ^{1}H NMR (400 MHz, (CD$_3$)$_2$CO) *δ* (ppm): 1.43 [s, 6H, 2CH$_3$ (Pbf)], 1.79 [m, 4H, β-H and γ-H (Arg)], 2.03 [s, 3H, CH$_3$ (Pbf)], 2.49 [s, 3H, CH$_3$ (Pbf)], 2.57 [s, 3H, CH$_3$ (Pbf)], 2.76 (dd, 1H, *J* = 11 and 14 Hz, *CH*$_2$-Ph), 2.96 [s, 2H, CH$_2$ (Pbf)], 3.07 [m, 2H, δ-H (Arg)], 3.12 [m, 1H, 3-H], 3.38 (dd, 1H, *J* = 4.5 and 12.5 Hz, 6-H), 3.48 [m, 1H, 4-CH$_2$ (Bn)], 3.49 (m, 1H, *CH*$_2$-Ph), 3.50 (m, 1H, 5-H), 3.63 (t, 1H, *J* = 12.5 Hz, 6-H), 4.18 [m, 2H, CH$_2$ (Bn, Urea)], 4.20 [m, 1H, 4-CH$_2$ (Bn)], 4.28 (m, 1H, 5-CH), 4.53 [d, 1H, *J* = 15 Hz, 1-CH$_2$ (Bn)], 4.68 [d, 1H, *J* = 15 Hz, 1-CH$_2$ (Bn)], 5.59 (d, 1H, *J* = 9 Hz, 5-CH-*NH*), 5.77 (t, 1H, *J* = 6 Hz, CO*NH*), 6.50 [br s, 3H, NHC(NH$_2$)=N], 7.04-7.43 (m, 20H, Ar); ^{13}C NMR (100 MHz, (CD$_3$)$_2$CO) *δ* (ppm): 12.6, 18.2, 19.5 [3CH$_3$ (Pbf)], 27.1 [C$_\gamma$ (Arg)], 28.7 [C$_\beta$ (Arg) and 2CH$_3$ (Pbf)], 38.6 (*CH*$_2$-Ph), 41.3 [C$_\delta$ (Arg)], 43.6 [CH$_2$ (Pbf)], 44.1 [CH$_2$ (Bn, Urea)], 46.2 (C$_6$), 50.4 [1-CH$_2$ (Bn)], 51.8 [4-CH$_2$ (Bn)], 52.5 (C$_5$-*CH*), 55.8 (C$_5$), 62.1 (C$_3$), 86.9, 117.5, 125.3 [3C (Pbf)], 126.8, 127.3,

128.1, 128.7, 128.9, 129.0, 129.3, 129.4, 129.9, 130.3 [20CH (Ar)], 132.8, 135.5 [2C (Pbf)], 138.7 [C (Ar)], 138.8 [C (Pbf)], 139.6, 140.3, 141.8 [3C (Ar)], 157.4 [C ($NHC(NH_2)=N$)], 158.6 [CO (Urea)], 158.9 [C (Pbf)], 171.0 (C_2); ES-MS *m/z* 885.0 $[M+1]^+$; Anal. calcd. for $C_{51}H_{61}N_7O_5S$: C, 69.28; H, 6.95; N, 11.09. Found: C, 69.54; H, 7.12; N, 10.86.

*4.8.4. (5*S*,3*S*)-1,4-Dibenzyl-5-((*S*)-1-(3-benzylureido)-2-phenylethyl)-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino) propyl)-2-oxopiperazine [**(S)-29a**]*

Amorphous solid (75 mg, 85%); $[\alpha]_D^{20}$ +9.9 (*c* 1.2, CH_2Cl_2); HPLC [Sunfire C_{18} (4.6 × 150 mm, 3.5 μm), 60-100% gradient of solvent A in B, 30 min] t_R 11.52 min; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm): 1.43 [s, 6H, $2CH_3$ (Pbf)], 1.54 [m, 2H, γ-H (Arg)], 1.70 [m, 2H, β-H (Arg)], 2.04 [s, 3H, CH_3 (Pbf)], 2.49 [s, 3H, CH_3 (Pbf)], 2.56 (m, 1H, *CH_2*-Ph), 2.57 [s, 3H, CH_3 (Pbf)], 2.98 (m, 1H, *CH_2*-Ph), 2.99 [s, 2H, CH_2 (Pbf)], 3.08 [m, 2H, δ-H (Arg)], 3.10 [m, 1H, 3-H and 5-H], 3.45 (d, 2H, *J* = 8 Hz, 6-H), 3.75 [d, 1H, *J* = 13.5 Hz, 4-CH_2 (Bn)], 3.99 [d, 1H, *J* = 13.5 Hz, 4-CH_2 (Bn)], 4.15 (m, 1H, 5-CH), 4.18 [dd, 1H, J = 5.5 and 15 Hz, CH_2 (Bn, Urea)], 4.27 [dd, 1H, J = 6.5 and 15 Hz, CH_2 (Bn, Urea)], 4.51 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 4.68 [d, 1H, *J* = 15 Hz, 1-CH_2 (Bn)], 5.75 (d, 1H, *J* = 9 Hz, 5-CH-*NH*), 5.97 (m, 1H, CO*NH*), 6.40 [br s, 3H, $NHC(NH_2)=N$], 7.07-7.45 (m, 20H, Ar); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 12.6, 18.3, 19.5 [$3CH_3$ (Pbf)], 26.9 [C_γ (Arg)], 28.7 [$2CH_3$ (Pbf)], 31.4[C_β (Arg)], 38.4 (*CH_2*-Ph), 41.0 [C_δ (Arg)], 43.7 [CH_2 (Pbf)], 44.2 [CH_2 (Bn, Urea)], 45.8 (C_6), 49.8 [1-CH_2 (Bn)], 54.9 (C_5-*CH*), 63.1 [4-CH_2 (Bn)], 63.6 (C_5), 63.8 (C_3), 87.0, 117.5, 125.4 [3C (Pbf)], 126.9, 127.4, 127.8, 128.1, 128.2, 128.7, 129.0, 129.1, 129.2, 129.5, 130.1, 130.3 [20CH (Ar)], 132.8, 135.7 [2C (Pbf)], 138.6 [C (Ar)], 138.7 [C (Pbf)], 140.0, 141.6 [3C (Ar)], 157.5 [C ($NHC(NH_2)=N$)], 158.9 [C (Pbf)], 159.9 [CO (Urea)], 172.1 (C_2); ES-MS *m/z* 885.0 $[M+1]^+$; Anal. calcd. for $C_{51}H_{61}N_7O_5S$: C, 69.28; H, 6.95; N, 11.09. Found: C, 69.03; H, 7.21; N, 10.97.

*4.9. General procedure for the synthesis of the Arg derived α-amino nitriles **(RS)-32a** and -**33a***

TEA (279 μL, 2 mmol mmol) was added to a solution of H-Arg(Pbf)-OMe·HCl (954 mg, 2 mmol) in MeOH (25 mL). After 15 min of stirring at rt, $ZnCl_2$ (272 mg, 2 mmol) was added, followed by the addition of the corresponding aldehyde **30** (295mg, 2.2 mmol) or **31** [57, 58] (601 mg, 2.2 mmol, freshly prepared as indicated in the Supplementary content) and the

mixture was stirred for 1 h. Then, TMSCN was added (375 μL, 3 mmol) and the mixture was stirred overnight at rt. The solvent was removed under reduced pressure and the residue was processed as above indicated in the general procedure for the synthesis of Ψ[CH(CN)NH] pseudodipeptides to give the α-amino nitriles **(*RS*)-32a** and ***-33a***.

4.9.1. N-*((*RS*)-1-Cyano-3-phenylpropyl)-Arg(Pbf)-OMe [(***RS)-32a****]*

White solid (992 mg, 85 %)); HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 μm), 10-100% gradient of solvent A in B, 5 min] t_R 5.52 min; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.45 [s, 6H, $2CH_3$ (Pbf)], 1.49-1.85 [m, 4H, β-H and γ-H (Arg)], 1.95-2.14 (m, 2H, 3-H), 2.03 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.56 [s, 3H, CH_3 (Pbf)], 2.68-2.89 (m, 2H, 4-H), 2.94 [s, 2H, CH_2 (Pbf)], 3.08-3.24 [m, 2H, δ-H (Arg)], 3.32 and 3.43 [2t, 1H, J = 6 Hz, α-H (Arg)], 3.46 and 3.55 (2t, 1 H, J = 6 Hz, 2-H), 3.71 and 3.74 (2s, 3H, OMe), 5.81 and 5.99 (br s, 3H, $NHC(NH_2)=N$), 7.14-7.31 (m, 5H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 12.6, 18.1, 19.4 [$3CH_3$ (Pbf)], 25.7 and 25.9 [C_γ (Arg)], 28.7 [$2CH_3$ (Pbf)], 30.5 and 30.8 [C_β (Arg)], 31.7 (C_4), 35.5 and 35.8 (C_3), 40.9 and 43.3 [CH_2 (Pbf)], 48.7 and 48.9 (C_2), 52.6 (OMe), 58.8 and 59.7 [C_α (Arg)], 86.6, 117.7, 124.8 [3C (Pbf)], 120.1 (CN), 126.6, 128.5, 128.8 (5 CH, Ph), 132.3, 132.9 (2C, Ph), 138.4, 139.9, 158.9 [3C (Pbf)], 156.3[C ($NHC(NH_2)=N$)], 174.3, 174.5 (CO_2); ES-MS *m/z* 583.2828 $[M+1]^+$; Anal. calcd. for $C_{30}H_{41}N_5O_5S$: C, 61.73; H, 7.08; N, 12.00. Found: C, 62.03; H, 7.21; N, 11.87.

4.9.2. N-*((RS)-3-(1-(*tert-*Butoxycarbonyl)-indol-3-yl)-1-cyanopropyl)-Arg(Pbf)-OMe [(***RS)-33a****]*

White solid (751 mg, 52 %)); HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 μm), 10-100% gradient of solvent A in B, 5 min] t_R 6.29 min; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.44 [s, 6H, $2CH_3$ (Pbf)], 1.67 (s. 9H, Boc), 1.53-1.89 [m, 4H, β-H and γ-H (Arg)], 2.08 [s, 3H, CH_3 (Pbf)], 2.17 (m, 2H, 3-H), 2.51 [s, 3H, CH_3 (Pbf)], 2.57 [s, 3H, CH_3 (Pbf)], 2.80-3.05 (m, 2H, 4-H), 2.93 [s, 2H, CH_2 (Pbf)], 3.07-3.27 [m, 2H, δ-H (Arg)], 3.32 and 3.48 [m, 1H, α-H (Arg)], 3.47 and 3.64 (m, 1 H, 2-H), 3.69 and 3.74 (2s, 3H, OMe), 5.71 and 5.96 [br s, 3H, $NHC(NH_2)=N$], 7.21-7.41 (m, 4H, Ind), 7.51 [d, 1H, J = 8 Hz, 4-H (Ind)]; ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 12.6, 18.1, 19.4 [$3CH_3$ (Pbf)], 20.09 and 21.1 (C_4), 25.3 and 25.5 [C_γ (Arg)], 28.4 [$3CH_3$ (Boc)], 28.7 [$2CH_3$ (Pbf)], 30.0 and 30.3 [C_β (Arg)], 33.1 and 33.2 (C_3), 41.1 [C_δ (Arg)], 43.3 [CH_2 (Pbf)], 48.5 and 48.9 (C_2), 52.4 and 52.5 (OMe), 58.7 and 59.4 [C_α

(Arg)], 83.9 (C, Boc), 86.6, 115.5, 123.1 [3C (Pbf)], 115.5 (C_3, Ind), 118.9 and 119.1 (CN), 118.6, 118.7, 119.0, 119.1, 119.9, 120.2, 122.7, 122.9, 124.8, 124.9 (CH, Ind), 130.2, 130.3 135.6 (C_{3a} and C_{7a}, Ind), 132.6, 138.7, 155.9 [3C (Pbf)], 159.0 [C (NHC(NH_2)=N)], 174.2 (CO_2); ES-MS *m/z* 722.3476 $[M+1]^+$; Anal. calcd. for $C_{37}H_{50}N_6O_7S$: C, 61.47; H, 6.97; N, 11.63. Found: C, 61.63; H, 7.11; N, 11.47.

4.10. General procedure for the synthesis of the 2-oxopiperazine derivatives ***(R)-*** *and* ***(S)-34a*** *and* ***-35a***

Raney-Ni (2.4 g, previously washed with MeOH) and hydrazine monohydrate (1.26 mL, 26 mmol) were added to a solution of the corresponding epimeric mixture of α-amino nitrile **(*RS*)-32a** and **-33a** (2.06 mmol) in MeOH (50 mL) and the mixture was heated at 65ºC for 20 min. Afterward, the reaction mixture was filtered over celite and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography, by using MeOH in CH_2Cl_2 gradient as mobile phase to obtain the desired resolved 2-oxopiperazines **(*R*)-** and **(*S*)-34a** and **-35a**.

*4.10.1. (5*R*,3*S*)-3-(3-(2-((2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-guanidino)propyl)-5-phenethyl-2-oxopiperazine [(**R**)-34a]*

White solid (345 mg, 31 %)); $[\alpha]_D^{20}$ -0.4 (*c* 2, $CHCl_3$); Mp: 129-131 ºC; HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 μm), 10-100% gradient of solvent A in B, 5 min] t_R 3.80 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.37 [s, 6H, $2CH_3$ (Pbf)], 1.53-1.91 [m, 6H, 1-H (phenethyl), β-H and γ-H (Arg)], 2.00 [s, 3H, CH_3 (Pbf)], 2.09 (br s, 1H, 4-H), 2.43 [s, 3H, CH_3 (Pbf)], 2.49 [s, 3H, CH_3 (Pbf)], 2.57 and 2.64 [2m, 2H, 2-H (phenethyl)], 2.85 [s, 2H, CH_2 (Pbf)], 2.88 (m, 1H, 5-H), 2.99-3.24 [m, 4H, 6-H and δ-H (Arg)], 3.31 (m, 1H, 3-H), 6.32 (br s, 1H, 1-H), 6.43 and 6.89 [br s, 3H, NHC(NH_2)=N], 7.07-7.12 and 7.17-7.20 (2 m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 12.6, 18.1, 19.4 [$3CH_3$ (Pbf)], 26.1 [C_γ (Arg)], 28.7 [$2CH_3$ (Pbf)], 29.5 [C_β (Arg)], 32.3 [C_2 (phenethyl)], 35.0 [C_1 (phenethyl)], 41.0 [C_δ (Arg)], 43.4 [CH_2 (Pbf)], 46.6 (C_5), 48.1 (C_6), 53.3(C_3), 86.5, 117.5, 124.7 [3C (Pbf)], 126.2, 128.4, 128.6 (5 CH, Ph), 141.4 (C, Ph), 132.4, 133.2 , 138.4, 158.7 [4C (Pbf)], 156.7 [C (NHC(NH_2)=N)], 173.3 (C_2); ES-MS *m/z* 556.2950 $[M+1]^+$; Anal. calcd. for $C_{29}H_{41}N_5O_4S$: C, 62.68; H, 7.44; N, 12.60. Found: C, 62.83; H, 7.51; N, 12.47.

*4.10.2. (5*S*,3*S*)-3-(3-(2-((2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-guanidino)propyl)-5-phenethyl-2-oxopiperazine [**(S)-34a**]*

White solid (389 mg, 35 %)); $[\alpha]_D^{20}$ -0.4 (*c* 2, $CHCl_3$); Mp: 128-130 ºC; HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 μm), 10-100% gradient of solvent A in B, 5 min] t_R 3.83 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.44 [s, 6H, $2CH_3$ (Pbf)], 1.57-1.92 [m, 6H, 1-H (phenethyl), β-H and γ-H (Arg)], 2.07 [s, 3H, CH_3 (Pbf)], 2.49 [s, 3H, CH_3 (Pbf)], 2.56 [s, 3H, CH_3 (Pbf)], 2.67 [2m, 2H, 2-H (phenethyl)], 2.93 [s, 2H, CH_2 (Pbf)], 3.00 (m, 1H, 5-H), 3.18 [m, 4H, 6-H and δ-H (Arg)], 3.50 (m, 1H, 3-H), 6.28- 6.56 [br s, 3H, $NHC(NH_2)=N$], 6.86 (br s, 1H, 1-H), 7.14-7.20 and 7.24-7.28 (2 m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 12.6, 18.1, 19.5 [$3CH_3$ (Pbf)], 25.4 [C_γ (Arg)], 28.7 [$2CH_3$ (Pbf)], 29.9 [C_β (Arg)], 32.3 [C_2 (phenethyl)], 35.0 [C_1 (phenethyl)], 41.1 [C_δ (Arg)], 43.4 [CH_2 (Pbf)], 48.0 (C_6), 52.2 (C_5), 58.3(C_3), 86.5, 117.6, 124.7 [3C (Pbf)], 126.3, 128.4, 128.8 (5 CH, Ph), 141.2 (C, Ph), 132.4, 133.2, 138.4, 158.8 [4C (Pbf)], 156.5 [C ($NHC(NH_2)=N$)], 172.5 (C_2); ES-MS *m/z* 556.2953 $[M+1]^+$; Anal. calcd. for $C_{29}H_{41}N_5O_4S$: C, 62.68; H, 7.44; N, 12.60. Found: C, 62.81; H, 7.53; N, 12.55.

*4.10.3. (5*R*,3*S*)-5-(2-(1-(*tert*-Butoxycarbonyl)-indol-3-yl)ethyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [**(R)-35a**]*

White solid (361 mg, 26 %)); $[\alpha]_D^{20}$ -5.8 (*c* 2, $CHCl_3$); Mp: 124-126 ºC; HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 μm), 10-100% gradient of solvent A in B, 5 min] t_R 4.10 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.36 [s, 6H, $2CH_3$ (Pbf)], 1.58 (s, 9H, Boc), 1.50-1.90 [m, 6H, 1-H (phenethyl), β-H and γ-H (Arg)], 1.99 [s, 3H, CH_3 (Pbf)], 2.42 [s, 3H, CH_3 (Pbf)], 2.49 [s, 3H, CH_3 (Pbf)], 2.62-2.80 [2m, 2H, 2-H (phenethyl)], 2.83 [s, 2H, CH_2 (Pbf)], 2.97 (m, 1H, 5-H), 3.02-3.28 [m, 4H, 6-H and δ-H (Arg)], 3.37 (m, 1H, 3-H), 6.34 (br s, 1H, 1-H), 6.45 [br s, 3H, $NHC(NH_2)=N$], 7.14 [t, 1H, J = 7 Hz, 5-H (Ind)], 7.23 [t, 1H, J = 7 Hz, 6-H (Ind)], 7.28 [s, 1H, 2-H, (Ind)], 7.42 [d, 1H, J = 7 Hz, 7-H, (Ind)], 8.02 [br s, 1H, 4-H, (Ind)]; ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 12.6, 18.1, 19.4 [$3CH_3$ (Pbf)], 21.5 [C_2 (phenethyl)], 26.1 [C_γ (Arg)], 28.4 [$3CH_3$ (Boc)], 28.7 [$2CH_3$ (Pbf)], 29.5 [C_β (Arg)], 32.3[C_1 (phenethyl)], 40.9 [C_δ (Arg)], 43.3 [CH_2 (Pbf)], 46.6 (C_5), 48.0 (C_6), 55.4 (C_3), 83.8 (C, Boc), 86.5, 117.6, 120.2 [3C (Pbf)], 115.4, 119.1, 122.5, 124,6 (5 CH, Ind), 130.5, 133.2 (2C, Ind), 132.4, 138.5, 158.8 [3C (Pbf)], 150.0 (CO, Boc), 156.7 [C ($NHC(NH_2)=N$)], 173.3 (C_2); ES-

MS *m/z* 695.3601 $[M+1]^+$; Anal. calcd. for $C_{36}H_{50}N_6O_6S$: C, 62.22; H, 7.25; N, 12.09. Found: C, 62.41; H, 7.33; N, 11.92.

*4.10.4. (5*S*,3*S*)-5-(2-(1-(*tert*-Butoxycarbonyl)-indol-3-yl)ethyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [(**S**)-35a]*

White solid (278 mg, 20 %)); $[\alpha]_D^{20}$ -4.4 (*c* 2, $CHCl_3$); Mp: 134-136 ºC; HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 μm), 10-100% gradient of solvent A in B, 5 min] t_R 4.12 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.36 [s, 6H, $2CH_3$ (Pbf)], 1.59 (s, 9H, Boc), 1.57-1.86 [m, 6H, 1-H (phenethyl), β-H and γ-H (Arg)], 2.00 [s, 3H, CH_3 (Pbf)], 2.42 [s, 3H, CH_3 (Pbf)], 2.50 [s, 3H, CH_3 (Pbf)], 2.70 [m, 2H, 2-H (phenethyl)], 2.84 [s, 2H, CH_2 (Pbf)], 2.91-3.22 [m, 5H, 5-H, 6-H and δ-H (Arg)], 3.41 (m, 1H, 3-H), 6.26 [br s, 3H, NHC(NH_2)=N], 6.67 (br s, 1H, 1-H), 7.14 [t, 1H, J = 7 Hz, 5-H (Ind)], 7.23 [t, 1H, J = 7 Hz, 6-H (Ind)], 7.31 [s, 1H, 2-H, (Ind)], 7.43 [d, 1H, J = 7 Hz, 7-H, (Ind)], 8.03 [br s, 1H, 4-H, (Ind)]; ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 12.6, 18.1, 19.4 [$3CH_3$ (Pbf)], 21.4 [C_2 (phenethyl)], 25.4 [C_γ (Arg)], 28.4 [$3CH_3$ (Boc)], 28.7 [$2CH_3$ (Pbf)], 29.0 [C_β (Arg)], 32.9 [C_1 (phenethyl)], 41.0 [C_δ (Arg)], 43.3 [CH_2 (Pbf)], 48.0 (C_6), 52.3 (C_5), 58.3 (C_3), 83.8 (C, Boc), 86.5, 117.6, 120.1 [3C (Pbf)], 115.5, 119.0, 122.6, 124,6 (5 CH, Ind), 130.5, 133.1 (2C, Ind), 132.4, 138.5, 158.8 [3C (Pbf)], 150.0 (CO, Boc), 156.5 [C (NHC(NH_2)=N)], 172.3 (C_2); ES-MS *m/z* 695.3593 $[M+1]^+$; Anal. calcd. for $C_{36}H_{50}N_6O_6S$: C, 62.22; H, 7.25; N, 12.09. Found: C, 62.45; H, 7.27; N, 11.87.

*4.11. General procedure for the removal of the Pbf protecting group in (**R**)- and (**S**)-34a. Synthesis of (**R**)- and (**S**)-36a*

The corresponding protected 2-oxopiperazine derivative **(*R*)-** and **(*S*)-34a** (93 mg, 0.17 mmol) was dissolved in (95:2.5:2.5) TFA:H_2O:TIPS mixture (1 mL). After 24 h of stirring at rt, the solvents were evaporated to dryness and the residue was lyophilized to give quantitatively the respective deprotected derivative **(*R*)-** and **(*S*)-36a**.

*4.11.1. (5*R*,3*S*)-3-(3-Guanidino)propyl)-5-phenethyl-2-oxopiperazine [(**R**)-36a] trifluoroacetate*

Amorphous solid (89 mg, 100 %)); $[\alpha]_D^{20}$ -2.3 (*c* 1, DMSO); HPLC-MS [Sunfire C_{18} (4.6×50 mm, 3.5 μm), 2-30 % gradient of solvent A in B, 5 min] t_R 1.02 min; ^{1}H NMR (400

MHz, D_2O) δ (ppm): 1.54-2.08 [m, 6H, 1-H (phenethyl), β-H (Arg) and γ-H (Arg)], 2.59 [m, 2H, 2-H (phenethyl)], 3.05 [t, 2H, J = 6 Hz, δ-H (Arg)], 3.28 (t, 1H, J = 12 Hz, 6-H) 3.42 (m, 1H, 5-H), 3.49 (dd, 1H, J = 4 and 12 Hz, 6-H) , 3.84 (t, 1H, J = 6 Hz, 3-H), 7.09-7.22 (m, 5H, Ph); ^{13}C NMR (100 MHz, D_2O) δ (ppm): 24.4 [C_γ (Arg)], 26.3 [C_β (Arg)], 30.4[C_2 (phenethyl)], 31.3 [C_1 (phenethyl)], 40.5 [C_δ (Arg)], 42.3 (C_6), 52.5 (C_5), 56.3 (C_3), 126.8, 128.5, 129.0 (5CH, Ph), 140.2 (C, Ph), 156.9 [C (NHC(NH_2)=N)], 167.8 (C_2); ES-MS *m/z* 304.43 $[M+1]^+$.

*4.11.2. (5*S*,3*S*)-3-(3-Guanidino)propyl)-5-phenethyl-2-oxopiperazine [**(S)-36a**]*

Amorphous solid (90 mg, 100 %)); $[\alpha]_D^{20}$ -5.4 (*c* 1, DMSO); HPLC-MS [Sunfire C_{18} (4.6×50 mm, 3.5 μm), 2-30 % gradient of solvent A in B, 5 min] t_R 1.01 min; ^{1}H NMR (400 MHz, D_2O) δ (ppm): 1.53 [m, 2H, γ-H (Arg)], 1.68-1.9 [m, 2H, β-H (Arg)], 1.80-2.01 [m, 2H, 1-H (phenethyl)], 2.58 [m, 2H, 2-H (phenethyl)], 2.98 [m, 2H, δ-H (Arg)], 3.25 (dd, 1H, J = 9 and 15 Hz, 6-H) 3.41-3.51 (m, 2H, 5-H and 6-H), 3.85 (t, 1H, J = 7 Hz, 3-H), 7.06-7.19 (m, 5H, Ph); ^{13}C NMR (100 MHz, D_2O) δ (ppm): 24.7 [C_γ (Arg)], 26.8 [C_β (Arg)], 30.1 [C_2 (phenethyl)], 30.6 [C_1 (phenethyl)], 40.4 [C_δ (Arg)], 41.7 (C_6), 49.1 (C_5), 53.9 (C_3), 126.8, 128.6, 129.0 (5CH, Ph), 140.1 (C, Ph), 156.8 [C (NHC(NH_2)=N)], 167.9 (C_2); ES-MS *m/z* 304.36 $[M+1]^+$.

*4.12. General procedure for the N_4-benzylation of the 2-oxopiperazines **34a** and **35a**. Synthesis of the 4-benzyl-2-oxopiperazines **(R)-** and **(S)-(37a** and **38a)***

DIEA (190 μL, 1.1 mmol) and benzyl bromide (260 μL, 2.2 mmol) were added under argon a solution of the corresponding 2-oxopiperazine **(*R*)-** and **(*S*)-6a**,**b** and **-7a** (0.55 mmol) in anhydrous CH_3CN (5 mL) at 60 ºC. After 3 h of stirring, the solvent was removed under reduced pressure. The residue was processed as indicated for the benzylation of analogues **6a** and **7a**.

*4.12.1. (5*R*,3*S*)-4-Benzyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-guanidino)propyl)-5-phenethyl-2-oxopiperazine [**(R)-37a**]*

White solid (259 mg, 73 %)); $[\alpha]_D^{20}$ +0 (*c* 2, $CHCl_3$); Mp: 89-91 ºC; HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 μm), 10-100% gradient of solvent A in B, 5 min] t_R 5.51 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.32 [m, 1H, γ-H (Arg)], 1.37 [s, 6H, $2CH_3$ (Pbf)], 1.43-1.74 [m,

4H, 1-H (phenethyl), β-H and γ-H (Arg)], 1.85 [m, 1H, 1-H (phenethyl)], 2.00 [s, 3H, CH_3 (Pbf)], 2.42 [s, 3H, CH_3 (Pbf)], 2.49 [s, 3H, CH_3 (Pbf)], 2.63 and 2.72 [2m, 2H, 2-H (phenethyl)], 2.82 [m, 2H, δ-H (Arg)], 2.85 [s, 2H, CH_2 (Pbf)], 2.97 (m, 1H, 3-H), 3.11 (m, 2H, 5-H and 6-H), 3.23 (t, 1H, J = 12 Hz, 6-H), 3.30 and 3.84 (2 d, 2H, J =13 Hz, 4-CH_2), 6.22 and 6.27 [2br s, 4H, 1-H and NHC(NH_2)=N], 7.09-7.23 (m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 12.7, 18.0, 19.4 [3CH_3 (Pbf)], 25.7 [C_γ (Arg)], 28.8 [C_β (Arg)], 28.7 [2CH_3 (Pbf)], 30.3 [C_1 (phenethyl)], 32.4 [C_2 (phenethyl)], 40.7 [C_δ (Arg)], 43.4 [CH_2 (Pbf)], 43.1 (C_6), 49.7 (C_5), 50.8 (4-CH_2), 60.3 (C_3), 86.4, 117.5, 124.7 [3C (Pbf)], 126.3, 128.5, 128.6 (10CH, Ph), 141.4 (2C, Ph), 132.4, 133.2, 138.5, 158.7 [4C (Pbf)], 156.5 [C (NHC(NH_2)=N)], 173.3 (C_2); ES-MS *m/z* 646.72 $[M+1]^+$; Anal. calcd. for $C_{36}H_{47}N_5O_4S$: C, 66.95; H, 7.33; N, 10.84. Found: C, 67.06; H, 7.51; N, 10.70.

*4.12.2. (5*S*,3*S*)-4-Benzyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-guanidino)propyl)-5-phenethyl-2-oxopiperazine [**(S)-37a**]*

White solid (255 mg, 72 %)); $[\alpha]_D^{20}$ +0 (*c* 2, $CHCl_3$); Mp: 73-75 ºC; HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 μm), 10-100% gradient of solvent A in B, 5 min] t_R 5.54 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.44 [s, 6H, 2CH_3 (Pbf)], 1.54 [m, 1H, γ-H (Arg)], 1.70 [m, 3H, 1-H (phenethyl) and β-H], 1.92 [m, 1H, 1-H (phenethyl)], 2.08 [s, 3H, CH_3 (Pbf)], 2.49 [s, 3H, CH_3 (Pbf)], 2.55 [s, 3H, CH_3 (Pbf)], 2.55 and 2.65 [2m, 2H, 2-H (phenethyl)], 2.80 (m, 1H, 5-H), 2.82 [m, 2H, δ-H (Arg)], 2.93 [s, 2H, CH_2 (Pbf)], 3.03-3.23 [m, 4H, 3-H, 6-H and δ-H (Arg)], 3.40 (m, 1H, 6-H), 3.62 and 3.81 (2 d, 2H, J =13 Hz, 4-CH_2), 6.59 [2br s, 4H, 1-H and NHC(NH_2)=N], 7.09-7.30 (m, 10H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 12.7, 18.0, 19.4 [3CH_3 (Pbf)], 25.7 [C_γ (Arg)], 28.7 [2CH_3 (Pbf)], 30.6 [C_β (Arg)], 32.1 [C_2 (phenethyl)], 35.2 [C_1 (phenethyl)], 40.7 [C_δ (Arg)], 42.7 (C_6), 43.4 [CH_2 (Pbf)], 57.6 (C_5), 59.4 (4-CH_2), 62.1 (C_3), 86.5, 117.6, 124.7 [3C (Pbf)], 126.3, 128.4, 128.6, 129.1 (10CH, Ph), 139.2, 141.6 (2C, Ph), 132.5, 133.2, 138.5, 158.8 [4C (Pbf)], 156.7 [C (NHC(NH_2)=N)], 174.1 (C_2); ES-MS *m/z* 646.66 $[M+1]^+$; Anal. calcd. for $C_{36}H_{47}N_5O_4S$: C, 66.95; H, 7.33; N, 10.84. Found: C, 66.86; H, 7.41; N, 10.68.

*4.12.3. (5*R*,3*S*)-4-Benzyl-5-(2-(1-(*tert*-butoxycarbonyl)-indol-3-yl)ethyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [**(R)-38a**]*

White solid (341 mg, 79 %)); $[\alpha]_D^{20}$ -1.4 (*c* 2, $CHCl_3$); Mp: 105-107 ºC; HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 μm), 10-100% gradient of solvent A in B, 5 min] t_R 6.19 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.43 [s, 6H, $2CH_3$ (Pbf)], 1.66 (s, 9H, Boc), 1.60-1.90 [m, 6H, 1-H (phenethyl), β-H and γ-H (Arg)], 2.07 [s, 3H, CH_3 (Pbf)], 2.49 [s, 3H, CH_3 (Pbf)], 2.55 [s, 3H, CH_3 (Pbf)], 2.82 [2m, 2H, 2-H (phenethyl)], 2.91 [s, 2H, CH_2 (Pbf)], 2.96 [m, 2H, δ-H (Arg)],3.11 (m, 1H, 3-H), 3.18-3.48 (m, 3H, 6-H and 4-CH_2), 3.69 (m, 1H, 5-H), 3.92 (d, 1H, J = 13 Hz, 4-CH_2), 6.33 [br s, 3H, NHC(NH_2)=N], 7.20-7.35 (m, 9H, 1-H and Ar), 7.49 [d, 1H, J = 8 Hz, 7-H, (Ind)], 8.09 [d, 1H, J = 8 Hz, 4-H, (Ind)]; ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 12.6, 18.0, 19.4 [$3CH_3$ (Pbf)], 21.7 [C_2 (phenethyl)], 25.7 [C_γ (Arg)], 28.4 [$3CH_3$ (Boc)], 28.7 [$2CH_3$ (Pbf)], 29.4 [C_β (Arg)], 31.9 [C_1 (phenethyl)], 40.7 [C_δ (Arg)], 43.0 (C_6), 43.3 [CH_2 (Pbf)], 50.9 (4-CH_2), 54.2 (C_5), 60.6 (C_3), 83.8 (C, Boc), 86.4, 117.5, 120.1 [3C (Pbf)], 115.5, 119.0, 122.6, 124,5, 124.6, 127.4, 128.5, 128.8, (10 CH, Ar), 130.4, 133.3 (2C, Ind), 135.6 (C, Ph), 132.3, 138.3, 158.6 [3C (Pbf)], 149.9 (CO, Boc), 156.5 [C (NHC(NH_2)=N)], 173.3 (C_2); ES-MS *m/z* 786.03 $[M+1]^+$; Anal. calcd. for $C_{43}H_{56}N_6O_6S$: C, 65.79; H, 7.19; N, 10.71. Found: C, 65.92; H, 7.23; N, 10.82.

*4.12.4. (5*S*,3*S*)-4-Benzyl-5-(2-(1-(*tert*-butoxycarbonyl)-indol-3-yl)ethyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [(**S**)-38a]*

White solid (302 mg, 70 %)); $[\alpha]_D^{20}$ +1.2 (*c* 2, $CHCl_3$); Mp: 94-96 ºC; HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 μm), 10-100% gradient of solvent A in B, 5 min] t_R 6.15 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.36 [s, 6H, $2CH_3$ (Pbf)], 1.58 (s, 9H, Boc), 1.40-1.77 [m, 5H, 1-H (phenethyl), β-H and γ-H (Arg)], 1.92 [m, 1H, 1-H (phenethyl)], 2.00 [s, 3H, CH_3 (Pbf)], 2.41 [s, 3H, CH_3 (Pbf)], 2.48 [s, 3H, CH_3 (Pbf)], 2.63 [m, 2H, 2-H (phenethyl)], 2.83 [s, 2H, CH_2 (Pbf)], 2.96-3.20 [m, 4H, 3-H, 6-H, δ-H (Arg)], 3.37 (m, 1H, 6-H), 3.59 (m, 2H, 5-H and 4-CH_2), 3.77 (br s, 1H, 4-CH_2), 6.26-6.91 [br s, 4H, 1-H, NHC(NH_2)=N], 7.11-7.24 (m, 8H, Ar), 7.35 [d, 1H, J = 8 Hz, 7-H, (Ind)], 8.02 [br s, 1H, J = 8 Hz, 4-H, (Ind)]; ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 12.6, 17.9, 19.4 [$3CH_3$ (Pbf)], 21.2 [C_2 (phenethyl)], 25.6 [C_γ (Arg)], 28.3 [$2CH_3$ (Pbf)], 28.6 [$3CH_3$ (Boc)], 30.2 [C_β (Arg)], 32.8 [C_1 (phenethyl)], 40.6 [C_δ (Arg)], 42.6 (C_6), 43.2 [CH_2 (Pbf)], 53.9 (C_5), 59.3 (4-CH_2), 62.1 (C_3), 83.5 (C, Boc), 86.4, 117.4, 120.3 [3C (Pbf)], 115.3, 118.9, 122.5, 124,4, 124.6, 127.4, 128.4, 128.8, (10 CH, Ar), 130.4, 133.2 (2C, Ind), 135.5 (C, Ph), 132.3, 138.4, 158.6 [3C (Pbf)], 149.8 (CO, Boc), 156.7

[C (NHC(NH_2)=N)], 174.1 (C_2); ES-MS *m/z* 785.95 $[M+1]^+$; Anal. calcd. for $C_{36}H_{50}N_6O_6S$: C, 65.79; H, 7.19; N, 10.71. Found: C, 65.94; H, 7.13; N, 10.65.

*4.13. General procedure for the benzylation of the 4-benzyl-2-oxopiperazines **37a** and **38a**. Synthesis of the 4-benzyl-2-oxopiperazines **(R)-** and **(S)-(39a**, **41a**, **42a** and **44a**)*

NaH (60% suspension in mineral oil, 9 mg, 0.21 mmol) and benzyl bromide (69 µL, 0.23 mmol) were added to a solution of the corresponding 2-oxopiperazine **37a** and **38a** (0.21 mmol) in anhydrous mixture THF/DMF (9:1, 5 mL) under argon at 0 ºC. After 24h of stirring, the crude reaction mixture was diluted with EtOAc (10 mL) and the excess of NaH was hydrolysed by addition of H_2O (2 mL). The aqueous layer was extracted with EtOAc (3×5 mL) and the organic extracts were dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography, using 30-100 EtOAc gradient in hexane as mobile phase to give the respective 1,4-dibenzyl-2-oxopiperazines **39a** and **42a**, as solids, in 20-42 % yield and the respective derivative benzylated at the guanidino group **41a** and **44a** as (*Z*/*E*)-isomeric mixtures in 8-14 % yield. These tribenzylated derivatives were dissolved in CH_3CN/H_2O (1:2, 2 mL) and the solutions were lyophilized to obtain amorphous solids.

*4.13.1. (5*R,*3*S*)-1,4-Dibenzyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)-sulfonyl)-guanidino)propyl)-5-phenethyl-2-oxopiperazine [**(R)-39a**]*

White solid (65 mg, 42 %)); $[\alpha]_D^{20}$ -3.1 (*c* 1, $CHCl_3$); Mp: 93-95 ºC; HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 µm), 10-100% gradient of solvent A in B, 5 min] t_R 6.36 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.42 [s, 6H, $2CH_3$ (Pbf)], 1.55 [m, 2H, γ-H (Arg)], 1.64 and 1.86 [2m, 2H, 1-H (phenethyl)], 1.71 [m, 2H, β-H (Arg)], 2.05 [s, 3H, CH_3 (Pbf)], 2.48 and 2.54 [2s, 6H, CH_3 (Pbf)], 2.54 and 2.62 [2m, 2H, 2-H (phenethyl)], 2.89 [m, 4H, δ-H (Arg) and CH_2 (Pbf)], 3.03-3.24 (m, 4H, 3-H, 5-H, 6-H), 3.34 and 3.83 (2d, 2H, J =12 Hz, 4-CH_2), 4.42 and 4.62 (2d, 2H, J =14 Hz, 1-CH_2), 6.97 and 6.08 [2br s, 3H, NHC(NH_2)=N], 7.02-7.34 (m, 15H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 12.6, 18.0, 19.4 [$3CH_3$ (Pbf)], 25.7 [C_γ (Arg)], 28.1 [C_β (Arg)], 28.7 [$2CH_3$ (Pbf)], 29.8 [C_1 (phenethyl)], 32.3 [C_2 (phenethyl)], 40.5 [C_δ (Arg)], 43.3 [CH_2 (Pbf)], 47.4 (C_6), 50.2 (C_5 and 1-CH_2), 51.3 (4-CH_2), 61.0 (C_3), 86.4, 117.5, 124.6 [3C (Pbf)], 126.2 and 127.9-129.2 (15CH, Ph), 136.6 and 141.4 (3C, Ph), 132.4, 133.2, 138.5, 158.7 [4C (Pbf)], 156.2 [C (NHC(NH_2)=N)], 171.0 (C_2); ES-MS *m/z* 736.70 $[M+1]^+$; Anal. calcd. for $C_{43}H_{53}N_5O_4S$: C, 70.17; H, 7.26; N, 9.52. Found: C, 70.29; H, 7.31; N, 9.36.

*4.13.2. (5S,3S)-1,4-Dibenzyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)-sulfonyl)-guanidino)propyl)-5-phenethyl-2-oxopiperazine [**(S)-39a**]*

White solid (60 mg, 40 %)); $[\alpha]_D^{20}$ +1.7 (*c* 1, $CHCl_3$); Mp: 70-72 ºC; HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 μm), 10-100% gradient of solvent A in B, 5 min] t_R 6.20 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.36 [s, 6H, $2CH_3$ (Pbf)], 1.39-1.65 [m, 5H, β-H, γ-H (Arg), and 1-H (phenethyl)], 1.75 [m, 1H, 1-H (phenethyl)], 1.98 [s, 3H, CH_3 (Pbf)], 2.34 [2m, 2H, 2-H (phenethyl)], 2.43 and 2.49 [2s, 6H, CH_3 (Pbf)], 2.67 (m, 1H, 5-H), 2.83 [s, 2H, CH_2 (Pbf)], 2.95 [m, 2H, δ-H (Arg)], 3.05 (dd, 1H, J = 8 and 13 Hz, 6-H), 3.22 (m, 2H, 3-H, 6-H), 3.57 and 3.65 (2d, 2H, J =14 Hz, 4-CH_2), 4.38 and 4.52 (2d, 2H, J =14.5 Hz, 1-CH_2), 6.00 and 6.09 [2br s, 3H, NHC(NH_2)=N], 6.83 (d, 2H, J = 7 Hz, Ph), 7.06-7.24 (m, 13H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 12.6, 18.0, 19.4 [$3CH_3$ (Pbf)], 25.6 [C_γ (Arg)], 28.7 [$2CH_3$ (Pbf)], 29.8 [C_β (Arg)], 30.6 [C_1 (phenethyl)], 32.1 [C_2 (phenethyl)], 40.8 [C_δ (Arg)], 43.3 [CH_2 (Pbf)], 47.4 (C_6), 50.1 (1-CH_2), 56.7 (C_5), 58.7 (4-CH_2), 61.5 (C_3), 86.4, 117.5, 124.6 [3C (Pbf)], 126.1 and 127.9-129.0 (15CH, Ph), 136.5 and 141.2 (3C, Ph), 132.4, 133.3, 138.4, 158.6 [4C (Pbf)], 156.2 [C (NHC(NH_2)=N)], 171.2 (C_2); ES-MS *m/z* 736.74 $[M+1]^+$; Anal. calcd. for $C_{43}H_{53}N_5O_4S$: C, 70.17; H, 7.26; N, 9.52. Found: C, 70.09; H, 7.27; N, 9.43.

*4.13.3. (5R,3S)-1,4-Dibenzyl-3-(3-(3-benzyl-2-((2,2,4,6,7-pentamethyl-2,3-dihydro-benzofuran-5-yl)sulfonyl)-guanidino)propyl)-5-phenethyl-2-oxopiperazine [**(R)-41a**]*

Amorphous solid (22 mg, 13 %)); HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 μm), 10-100% gradient of solvent A in B, 5 min] t_R 5.66 (83%) and 5.85 (17%) min; ^{1}H NMR [500 MHz, $(CD_3)_2CO$] δ (ppm): 1.41 and 1.42 [2s, 6H, $2CH_3$ (Pbf)], 1.57-1.78 [m, 4H, β-H and γ-H (Arg)], 1.71 and 1.94 [2m, 2H 1-H (phenethyl)], 2.01 [s, 3H, CH_3 (Pbf)], 2.46 [s, 3H, CH_3 (Pbf)], 2.52 [s, 3H, CH_3 (Pbf)], 2.60 and 2.75 [2m, 2H, 2-H (phenethyl)], 2.93 [s, 2H, CH_2 (Pbf)], 3.07 [m, 3H, 3-H and δ-H (Arg)], 3.19 (bd, 1H, J = 10 Hz, 6-H), 3.26 (m, 1H, 3-H), 3.36 (m, 1H, 6-H), 3.39 (d, 1H, J = 14 Hz, 4-CH_2), 3.94 (m, 1H, 4-CH_2), 4.43 [d, 2H, J = 5 Hz, NHC(NH-*CH_2*)=N], 4.53 and 4.63 (2 d, 2H, J = 14.5 Hz, 1-CH_2), 7.10-7.37 (m, 20H, Ph); ^{13}C NMR [125 MHz, $(CD_3)_2CO$] δ (ppm): 12.5, 18.3, 19.6 [$3CH_3$ (Pbf)], 26.7 [C_γ (Arg)], 28.6 [C_β (Arg)], 28.7 [$2CH_3$ (Pbf)], 30.3 [C_1 (phenethyl)], 33.0 [C_2 (phenethyl)], 41.6 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 45.3 (NHC(NH-*CH_2*)=N), 48.1 (C_6), 50.2 (1-CH_2), 51.0 (C_5), 51.5 (4-CH_2), 61.9 (C_3), 86.9, 117.5, 125.2 [3C (Pbf)], 126.6, 127.9-129.9 (20CH, Ph), 142.7 and 139.9 (4C, Ph), 132.7, 135.6, 138.7, 158.7 [4C (Pbf)], 155.9 [NHC(NH-Bn)=N)], 170.5 (C_2); ES-MS *m/z*

827.04 $[M+1]^+$; Anal. calcd. for $C_{50}H_{59}N_5O_4S$: C, 72.70; H, 7.20; N, 8.48. Found: C, 72.89; H, 7.31; N, 8.25.

*4.13.4. (5*S*,3*S*)-1,4-Dibenzyl-3-(3-(3-benzyl-2-((2,2,4,6,7-pentamethyl-2,3-dihydro-benzofuran-5-yl)sulfonyl)-guanidino)propyl)-5-phenethyl-2-oxopiperazine [**(S)-41a**]*

Amorphous solid (24 mg, 14 %)); HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 μm), 10-100% gradient of solvent A in B, 5 min] t_R 5.63 (70%) and 5.84 (30%) min; ^{1}H NMR [500 MHz, $(CD_3)_2CO$] δ (ppm): 1.41 and 1.43 [2s, 6H, $2CH_3$ (Pbf)], 1.54-1.76 [m, 4H, β-H and γ-H (Arg)], 1.57 and 1.82 [2m, 2H 1-H (phenethyl)], 2.01 [s, 3H, CH_3 (Pbf)], 2.45 [s, 3H, CH_3 (Pbf)], 2.51 [s, 3H, CH_3 (Pbf)], 2.47 [m, 2H, 2-H (phenethyl)], 2.83 (m, 1H, 5-H), 2.95 [s, 2H, CH_2 (Pbf)], 3.02-3.16 [m, 2H, δ-H (Arg)], 3.18-3.28 (m, 2H, 3-H and 6-H), 3.48 and 3.51 (2d, 1H, J = 5 and 13 Hz, 6-H), 3.67, 3.72 and 3.79 (3d, 2H, J = 14 Hz, 4-CH_2), 4.42 [d, 2H, J = 6 Hz, NHC(NH-*CH_2*)=N], 4.57 (s, 2H, 1-CH_2), 7.08-7.35 (m, 20H, Ph); ^{13}C NMR [125 MHz, $(CD_3)_2CO$] δ (ppm): 12.5, 18.3, 19.5 [$3CH_3$ (Pbf)], 26.7 [C_γ (Arg)], 28.7 [$2CH_3$ (Pbf)], 31.1 [C_β (Arg)], 32.8 [C_2 (phenethyl)], 35.8 [C_1 (phenethyl)], 41.8 [C_δ (Arg)], 43.6 [CH_2 (Pbf)], 45.3 (NHC(NH-*CH_2*)=N), 48.0 (C_6), 50.2 (1-CH_2), 57.5 and 57.7 (C_5), 59.3 (4-CH_2), 64.2 (C_3), 86.9, 117.5, 125.2 [3C (Pbf)], 126.5, 127.9-129.9 (20CH, Ph), 140.4, 140.5 and 142.8 (4C, Ph), 132.8, 135.6, 138.7, 158.8 [4C (Pbf)], 155.9 [NHC(NH-Bn)=N)], 171.1 (C_2); ES-MS *m/z* 827.04 $[M+1]^+$; Anal. calcd. for $C_{50}H_{59}N_5O_4S$: C, 72.70; H, 7.20; N, 8.48. Found: C, 72.87; H, 7.33; N, 8.31.

*4.13.5. (5*R*,3*S*)-1,4-Dibenzyl-5-(2-(1-(*tert*-butoxycarbonyl)-indol-3-yl)ethyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [**(R)-42a**]*

White solid (40 mg, 22 %)); $[\alpha]_D^{20}$ -8.4 (*c* 1, $CHCl_3$); Mp: 106-108 ºC; HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 μm), 80-100% gradient of solvent A in B, 5 min] t_R 4.06 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.44 [s, 6H, $2CH_3$ (Pbf)], 1.67 (s, 9H, Boc), 1.55-2.03 [m, 6H, 1-H (phenethyl), β-H and γ-H (Arg)], 2.07 [s, 3H, CH_3 (Pbf)], 2.51 [s, 3H, CH_3 (Pbf)], 2.57 [s, 3H, CH_3 (Pbf)], 2.47-2.62 and 2.64-2.78 [2m, 2H, 2-H (phenethyl)], 2.90 [s, 2H, CH_2 (Pbf)], 2.85-3.04 [m, 2H, δ-H (Arg)], 3.05-3.31 (m, 4H, 3-H, 5-H, 6-H), 3.38 and 3.81 (2d, 2H, J = 13 Hz, 4-CH_2), 4.42 and 4.69 (2d, 2H, J = 14 Hz, 1-CH_2), 6.04 and 6.19 [2br s, 3H, NHC(NH_2)=N], 7.12-7.24 (m, 13H, Ar), 7.32 [d, 1H, J = 7.5 Hz, 7-H, (Ind)], 8.01 [br s, 1H, 4-H, (Ind)]; ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 12.6, 18.0, 19.4 [$3CH_3$ (Pbf)], 21.7 [C_2

(phenethyl)], 25.7 [C_γ (Arg)], 28.4 [3CH_3 (Boc)], 28.7 [2CH_3 (Pbf)], 29.8 [C_β (Arg)], 31.9 [C_1 (phenethyl)], 40.6 [C_δ (Arg)], 43.3 [CH_2 (Pbf)], 47.5 (C_6), 50.2 (1-CH_2), 50.4 (C_5), 51.3 (4-CH_2), 61.1 (C_3), 83.8 (C, Boc), 86.3, 117.4, 124.6 [3C (Pbf)], 115.4, 118.9, 120.0, 122.5, 124.5, 127.6-129.0, 130.3, 135.5 (15 CH, Ar), 130.3, 133.2 (2C, Ind), 136.6, 138.4 (2C, Ph), 132.3, 135.6, 138.3, 158.6 [4C (Pbf)], 149.9 (CO, Boc), 156.2 [C (NHC(NH_2)=N)], 171.0 (C_2); ES-MS *m/z* 876.11 $[M+1]^+$; Anal. calcd. for $C_{50}H_{62}N_6O_6S$: C, 68.62; H, 7.14; N, 9.60. Found: C, 68.92; H, 7.25; N, 9.52.

*4.13.6. (5*S*,3*S*)-1,4-Dibenzyl-5-(2-(1-(*tert*-butoxycarbonyl)-indol-3-yl)ethyl-3-(3-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [**(S)-42a**]*

White solid (77 mg, 42 %)); $[\alpha]_D^{20}$ +7.7 (*c* 1, $CHCl_3$); Mp: 96-98 ºC; HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 μm), 40-100% gradient of solvent A in B, 5 min] t_R 6.43 min; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.46 [s, 6H, 2CH_3 (Pbf)], 1.68 (s, 9H, Boc), 1.53-1.99 [m, 6H, 1-H (phenethyl), β-H and γ-H (Arg)], 2.08 [s, 3H, CH_3 (Pbf)], 2.53 [s, 3H, CH_3 (Pbf)], 2.59 [s, 3H, CH_3 (Pbf)], 2.47-2.56 [m, 2H, 2-H (phenethyl)], 2.77 (m, 1H, 5-H), 2.79 [s, 2H, CH_2 (Pbf)], 2.96 [m, 2H, δ-H (Arg)], 3.08 (dd, 1H, J =8.5 and 13 Hz, 6-H), 3.19-3.28 (m, 2H, 3-H, and 6-H), 3.60 and 3.62 (2d, 2H, J = 14 Hz, 4-CH_2), 4.43 and 4.49 (2d, 2H, J = 14 Hz, 1-CH_2), 6.01 and 6.12 [2br s, 3H, NHC(NH_2)=N], 7.05-7.23 (m, 14H, Ar), 7.99 [br s, 1H, 4-H, (Ind)]; ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 12.6, 18.0, 19.4 [3CH_3 (Pbf)], 21.3 [C_2 (phenethyl)], 25.9 [C_γ (Arg)], 28.3 [3CH_3 (Boc)], 28.6 [2CH_3 (Pbf)], 29.8 [C_β (Arg)], 30.4 [C_1 (phenethyl)], 40.8 [C_δ (Arg)], 43.3 [CH_2 (Pbf)], 47.4 (C_6), 50.1 (1-CH_2), 56.8 (C_5), 58.8 (4-CH_2), 63.6 (C_3), 83.7 (C, Boc), 86.3, 117.4, 124.5 [3C (Pbf)], 115.3, 118.8, 120.0, 122.3, 122.5, 127.5-128.9 (15 CH, Ar), 130.3, 133.3 (2C, Ind), 135.5, 138.9 (2C, Ph), 132.3, 136.4, 138.4, 158.6 [4C (Pbf)], 149.8 (CO, Boc), 156.2 [C (NHC(NH_2)=N)], 171.2 (C_2); ES-MS *m/z* 876.11 $[M+1]^+$; Anal. calcd. for $C_{50}H_{62}N_6O_6S$: C, 68.62; H, 7.14; N, 9.60. Found: C, 68.82; H, 7.22; N, 9.47.

*4.13.7. (5*R*,3*S*)-1,4-Dibenzyl-5-(2-(1-(*tert*-butoxycarbonyl)-indol-3-yl)ethyl-3-(3-(3-benzyl-2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [**(R)-44a**]*

Amorphous solid (20 mg, 10 %)); HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 μm), isocratic 95 % of solvent A in 5% of B] t_R 2.32 (73%) and 2.47 (27%) min; ^{1}H NMR [500 MHz,

$(CD_3)_2CO$] δ (ppm): 1.39 and 1.42 [2s, 6H, $2CH_3$ (Pbf)], 1.64 (s, 9H, Boc), 1.55-2.03 [m, 6H, 1-H (phenethyl), β-H and γ-H (Arg)], 1.99 [s, 3H, CH_3 (Pbf)], 2.45 [s, 3H, CH_3 (Pbf)], 2.51 [s, 3H, CH_3 (Pbf)], 2.59-2.78 [m, 2H, 2-H (phenethyl)], 2.92 [s, 2H, CH_2 (Pbf)], 3.06 [m, 2H, δ-H (Arg)], 3.13 (m, 1H, 3-H), 3.20 and 3.38 (2m, 2H, 6-H), 3.34 (m, 1H, 5-H), 3.41 (d, 1H, J = 14 Hz, 4-CH_2), 3.91 (m, 1H, 4-CH_2), 4.42 [d, 2H, J = 6 Hz, NHC(NH-*CH_2*)=N], 4.53, 4.55, 4.60 and 4.65 (4d, 2H, J = 14 Hz, 1-CH_2), 7.12-7.37 (m, 18H, Ar), 7.49 and 7.53 (2d, 1H, J = 8 Hz, 7-H, (Ind)], 8.12 [d, 1H, J = 7.5 Hz, 4-H, (Ind)]; ^{13}C NMR [125 MHz, $(CD_3)_2CO$] δ (ppm): 12.4, 18.1, 19.4 [$3CH_3$ (Pbf)], 22.0 [C_2 (phenethyl)], 24.7 [C_γ (Arg)], 28.1 [$2CH_3$ (Pbf)], 28.5 [$3CH_3$ (Boc)], 27.6 [C_β (Arg)], 28.4 [C_1 (phenethyl)], 41.4 [C_δ (Arg)], 43.4 [CH_2 (Pbf)], 47.9 (C_6), 50.1 (1-CH_2), 51.2 (C_5), 51.5 (4-CH_2), 61.8 and 61.9 (C_3), 83.9 (C, Boc), 86.7, 117.3, 124.9 [3C (Pbf)], 115.8, 119.8, 121.2, 123.0, 124.9, 125.1, 127.7-129.6 (20 CH, Ar), 131.2, 135.5 (2C, Ind), 136.3, 138.5, 139.7 (3C, Ph), 132.6, 135.4, 138.7, 158.7 [4C (Pbf)], 150.1 (CO, Boc), 155.7 and 156.5 [NHC(NH-Bn)=N)], 170.6 (C_2); ES-MS *m/z* 965.96 $[M+1]^+$; Anal. calcd. for $C_{57}H_{68}N_6O_6S$: C, 70.93; H, 7.10; N, 8.71. Found: C, 70.82; H, 7.14; N, 8.82.

*4.13.8. (5*S*,3*S*)-1,4-Dibenzyl-5-(2-(1-(*tert*-butoxycarbonyl)-indol-3-yl)ethyl-3-(3-(3-benzyl-2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)propyl)-2-oxopiperazine [**(S)-44a**]*

Amorphous solid (16 mg, 8 %)); HPLC-MS [Sunfire C_{18} (4.6 × 50 mm, 3.5 μm), isocratic 95 % of solvent A in 5% of B] t_R 2.36 (75%) and 2.52 (25%) min; ^{1}H NMR [500 MHz, $(CD_3)_2CO$] δ (ppm): 1.41 [s, 6H, $2CH_3$ (Pbf)], 1.65 (s, 9H, Boc), 1.67-1.91 [m, 6H, 1-H (phenethyl), β-H and γ-H (Arg)], 2.00 [s, 3H, CH_3 (Pbf)], 2.43 [s, 3H, CH_3 (Pbf)], 2.51 [s, 3H, CH_3 (Pbf)], 2.58 [m, 2H, 2-H (phenethyl)], 2.91 [s, 2H, CH_2 (Pbf)], 2.96 (m, 1H, 5-H), 3.12 [m, 2H, δ-H (Arg)], 3.29 (m, 1H, 3-H), 3.27 and 3.59 (2m, 2H, 6-H), 3.78 (m, 1H, 4-CH_2), 4.42 and 4.56 [2d, 2H, J = 5.5 Hz, NHC(NH-*CH_2*)=N], 4.51 and 4.67 (2d, 2H, J = 14.5 Hz, 1-CH_2), 7.12-7.37 (m, 18H, Ar), 7.41 (d, 1H, J = 7.5 Hz, 7-H, (Ind)], 8.09 [d, 1H, J = 7.5 Hz, 4-H, (Ind)]; ^{13}C NMR [125 MHz, $(CD_3)_2CO$] δ (ppm): 12.2, 18.0, 19.3 [$3CH_3$ (Pbf)], 21.9 [C_2 (phenethyl)], 26.7 [C_γ (Arg)], 28.0 [$2CH_3$ (Pbf)], 28.3 [$3CH_3$ (Boc)], 31.0 [C_β (Arg)], 33.5 [C_1 (phenethyl)], 41.8 [C_δ (Arg)], 43.3 [CH_2 (Pbf)], 45.3 (1-CH_2), 48.0 (C_6), 57.6 (C_5), 59.2 (4-CH_2), 64.2 (C_3), 84.0 (C, Boc), 86.9, 117.5, 125.0 [3C (Pbf)], 115.9, 119.9, 121.4, 123.2, 125.0, 125.2, 127.9-129.8 (20 CH, Ar), 131.3, 135.7, 135.1 (2C, Ind), 136.4, 138.4, 140.5 (3C, Ph), 132.8, 135.6, 138.7, 158.8 [4C (Pbf)], 150.3 (CO, Boc), 155.9 and 156.5 [NHC(NH-

Bn)=N)], 171.2 (C_2); ES-MS *m/z* 965.96 $[M+1]^+$; Anal. calcd. for $C_{57}H_{68}N_6O_6S$: C, 70.93; H, 7.10; N, 8.71. Found: C, 71.07; H, 7.19; N, 8.60.

4.14. 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 ºC. At the beginning of each experiment, aggregation response to SFLLRN (30μM) 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.

4.15. Cytotoxicity assays

A colorimetric assay, using the sulforhodamine B (SRB) reaction, was adapted for a quantitative measurement of cell growth and viability, following the technique described by Skehan, P. A. *et al*. [59]. Cells (MDA-MB-231, A549 and HT-29) were seeded in 96 well microtiter plates, at 5×10^3 cells per well in aliquots of 195 μL of RPMI medium, and they were allowed to attach to the plate surface by growing in drug free medium for 18 h. Afterwards, samples were added in aliquots of 5 μL [dissolved in (3:7) DMSO/H_2O]. After 48 h exposure, cells were fixed by adding 50 μL of cold 50% (wt/vol) trichloroacetic acid, and incubating at 4 ºC for 60 min. Then, the plates were washed with deionized H_2O and dried. 100 μL of SRB solution (0.4% wt/vol in 1% acetic acid) was added to each microtiter well and these were incubated at room temperature for 10 min. Unbound SRB was removed by washing with 1% acetic acid, the plates were air dried, and the bound stain was solubilized with Tris buffer. Optical densities were read on an automated spectrophotometer plate reader at a single wavelength of 490 nm. Data analysis was automatically generated by the high

throughput screening LIMS implemented at the laboratory. The three response parameters GI_{50} (50% cell growth inhibition), LC_{50} (50% lethal concentration), and TGI (total growth inhibition) were extracted from concentration-response curves by linear interpolation, according to the National Cancer Institute (NCI) protocols [60].

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Supplementary Content Available: Synthesis procedure for the aldehyde **31** and ^{1}H and ^{13}C NMR spectra of all new compounds.

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