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Modulators of hERAP2 discovered by High-Throughput Screening.

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Keywords: enzymes; screening; inhibitors; activators; antigen presentation; metalloproteases

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Abstract :

Endoplasmic reticulum aminopeptidase 2, ERAP2, is an emerging pharmacological target in cancer immunotherapy and control of autoinflammatory diseases, as it is involved in antigen processing. It has been linked to the risk of the development of spondyloarthritis, and it associates with the immune infiltration of tumours and strongly predicts the overall survival for patients receiving check-point inhibitor therapy. While some selective inhibitors of its homolog ERAP1 are available, no selective modulator of ERAP2 have been disclosed so far. In order to identify such compounds, we screened an *in-house* focused library of 1920 compounds designed to target metalloenzymes. Structure-Activity Relationships and docking around two hits led to the discovery of selective inhibitors of ERAP2. Amid those, some bind to yet untapped amino-acids in the S1 pocket. Importantly, we disclose also the first activator of small substrates hydrolysis by ERAP2. Inhibitors and activators identified in this study could serve as useful starting points for optimization.

Abbreviations used

ACN acetonitrile; Arg-AMC L-Arginine-7-amido-4-methylcoumarin hydrochloride; DABA diaminobenzoic acid; DMF dimethylformamide; DMSO dimethylsulfoxide; ERAP1 Endoplasmic reticulum aminopeptidase 1; ERAP2 Endoplasmic reticulum aminopeptidase 2; HBTU 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium; Hepes 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid buffer; HOBt Hydroxybenzotriazole; HTS High-throughput screening; L-AMC L-Leucine-7-amido-4-methylcoumarin hydrochloride; Leu-AMC L-Leucine-7-amido-4-methylcoumarin hydrochloride; MHCI major histocompatibility complex class I; r.t room temperature; R-AMC L-Arginine-7-amido-4-methylcoumarin hydrochloride; SAR Structure activity relationships; SNP single nucleotide polymorphism; TEA triethylamine; TFA trifluoroacetic acid; THF tetrahydrofurane.

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1. Introduction

Endoplasmic reticulum aminopeptidase 2 (ERAP2) is a M1 family zinc metalloprotease playing a key role in antigen presentation pathway [1]. This intracellular aminopeptidase trims peptide precursors resulting from protein degradation by the proteasome and thereby generates mature antigenic epitopes of appropriate length for presentation on the cell surface by major histocompatibility complex class I (MHC-I) molecules [2]. The recognition of the extracellular peptide by cytotoxic T-cell triggers immune response against infected or diseased cells through biological cascades that lead to apoptosis of the target cell. Thereby ERAP2 is a major regulator of adaptive immune responses in humans. In line with this role, common single nucleotide polymorphisms (SNPs) in ERAP2 that affect protein expression or enzymatic activities have recently been associated with predisposition to immune diseases (i.e. ankylosing spondylitis) and infectious diseases [3]. Furthermore, cancer and pathogens can evade the immune system by stopping the generation of antigenic peptides [4,5].

These recent findings establishing the role of ERAP2 in autoimmunity, infections, and cancers have turned the spotlight on this aminopeptidase as pharmaceutical target [6,7]. Thus, it is of paramount importance to find biological tools that can either inhibit or enhance their action, whether for the better understanding of biological mechanisms implying ERAP2 or for the discovery of potential therapeutic compounds. One main challenge of targeting ERAP2 is the development of selective modulators. Indeed, ERAP1 is another major aminopeptidase highly homologous to ERAP2 with 50% sequence identity. These two enzymes process antigen peptides in a concerted and complementary manner [8,9] but display separate substrates specificity [10,11,12].

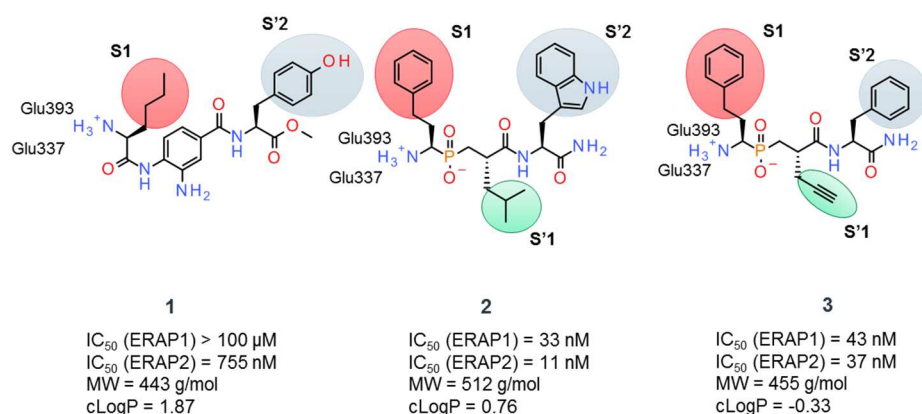
Recent co-crystal structures of the ERAPs with ligands bound in the active site reveal key differences that may help the rational design of selective modulators [13]. Among the common structural characteristics shared by all ERAPs that have been identified, there are the single Zinc(II) ion present in the catalytic site and coordinated by the HExxHx18E zinc-binding motif, as well as the GAMEN aminopeptidase motif responsible for free N-terminus recognition. Nevertheless, at least three specificity pockets, namely S1, S1' and S2', can determine substrate selectivity [14]. S1 specificity pocket is structurally rigid and contains a hydrophobic pocket conserved in both enzymes [11]. However, non-conserved residues like Glu177, Asp198, Gln447 in ERAP2 (His160, Gln181, Arg430 in ERAP1, respectively) induce a preference of ERAP2 for substrates with a positively charged N-terminal side chain [11,12]. The S1' specificity pocket shows a preference for small hydrophobic

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groups and S2' specificity pocket is a hydrophobic pocket having a residue Tyr892 unique to ERAP2 that enables additional π - π stacking interactions [12,14].

To date, only a few ERAP2 inhibitors have been identified. By using a rational, structure-based design, aiming to accommodate the specific pockets of these enzymes, Papakyriakou *et al.* reported a series of diaminobenzoic acid (DABA) derivatives with moderate potency [15,16]. Among them, the most selective compound **1** (IC_{50} ERAP2 = 0.755 μ M) exhibits a 130-fold selectivity against ERAP1 (Fig. 1). Another well described ERAP2 inhibitors family belongs to the series of pseudopeptides containing a phosphinic group. The best analog **2** reported by Zervoudi *et al.* displays nanomolar potency toward ERAP2 but lacks selectivity against ERAP1 (Fig. 1) [14]. Later, optimization by Kokkala *et al.* varying the side chains that occupy S1' and S2' pockets has identified inhibitors with comparable potency and moderate selectivity, like **3** (Fig. 3) [17]. The report by Węglarz-Tomczak *et al.* also on phosphinic series used the same strategy of structural improvements by modifying substituents that can further fit in the S1 and S1' subsites [18]. However, the described derivatives exhibit less potency for ERAP2. Overall, only two main scaffolds have been explored for the design of ERAP2 inhibitors and these compounds either lack selectivity against other aminopeptidases and/or show poor druggability properties.

Fig. 1: Example of hERAP2 inhibitors reported in the literature.^a



^a Substituents filling pockets S1, S'1 and S'2 are colored respectively in red, green and blue. cLogP was calculated using Datawarrior™

Thus, alternative approaches to discover new structures that could serve as starting points for optimization towards ERAP2 inhibitors is still challenging and highly needed. To fulfill the need of new chemical templates able to inhibit ERAP2, we chose to develop a fast, enzyme-efficient 384-well plate HTS assay, and applied it to a focused *in-house* library. This screening assay allowed us to discover structurally original inhibitors, but also, surprisingly, activators of small peptide hydrolysis by ERAP2.

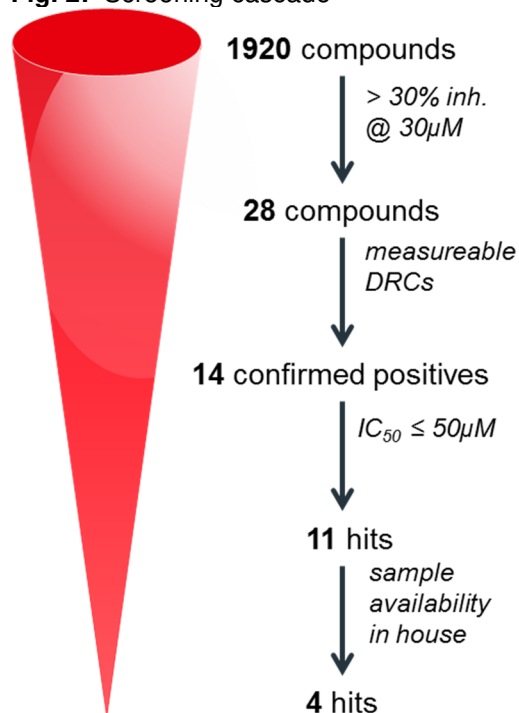
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2. Results and Discussion

2.1. Development of 384-well plate assay

The bioassay used in this study is based on the enzymatic conversion of a fluorogenic substrate, Arg-aminomethylcoumarine (Arg-AMC) ($K_m=16\mu\text{M}$) into a fluorescent product 7-amino-4-methylcoumarin ($\lambda_{\text{ex}} = 380 \text{ nm}$; $\lambda_{\text{em}} = 450 \text{ nm}$). Several parameters (V/well, buffer, vehicle ...) were pre-fixed, but the nature and concentration of the substrate, as well as the concentration of the enzyme remained to be set. Arg-AMC is more selective of ERAP2 (compared to Leu-AMC), with a K_m of $16 \mu\text{M}$, and was thus used at a concentration of $5 \mu\text{M}$. Several concentrations of the enzyme were then tested, and we chose to work with $0.5 \mu\text{g/mL}$ of ERAP2. This appeared to be the best compromise between intensity of the signal, and stability or reproducibility, expressed by the Z' factor ($Z' = 0.6$ for the whole plate), to avoid plate effect at lower concentration. (Supplementary Fig. S1)

Fig. 2: Screening cascade^a



^a DRCs: Dose-Response Curves.

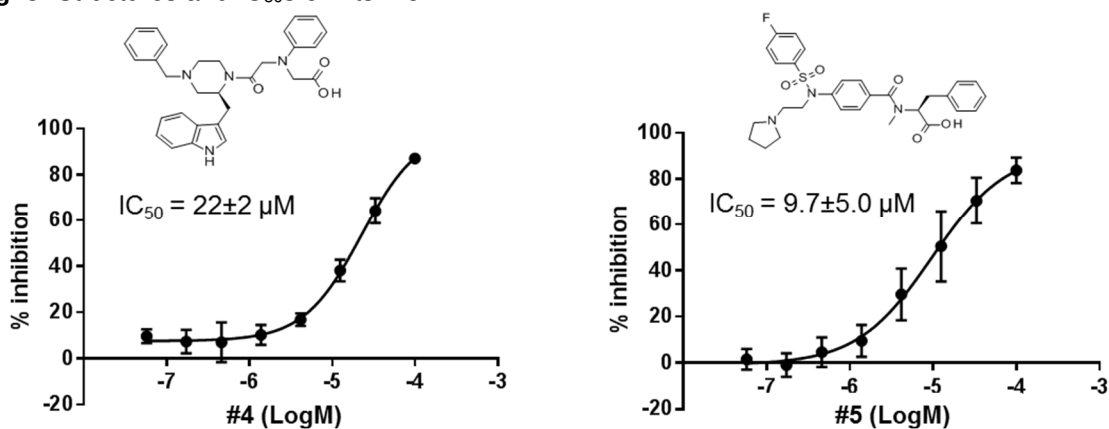
2.2. Screening of a 1920-member library.

Figure 2 summarizes the screening cascade. An *in-house* library of 1920 drug-like carboxylic acids and bioisosters, aiming at targeting metalloenzymes, was distributed in plates using nanoacoustic dispensing and screened in this fluorescence assay (Supplementary Fig. S2). [19] Bestatine at $100\mu\text{M}$ was used as a positive control, and displayed stable activity throughout the screening ($Z' > 0.5$ for

[Tapez ici]

each plate) (Supplementary Fig. S3 and S4). Out of the 28 compounds that displayed an inhibition above 30% at 30 μM , half were confirmed (dose-dependent inhibition of ERAP2) and 11 compounds had a measured IC_{50} below 50 μM and were defined as hits (**4-14**). Their IC_{50} values range from 9 μM to 28 μM . Among these hits (Supplementary Fig. S5), 3 displayed a carboxylic acid as putative zinc binding group, and a singleton bore a tetrazole. More interestingly, 7 compounds with IC_{50} values around 15 μM displayed less common zinc-binding groups like thio-five-membered ring, either oxa-2,4-diazol, oxa-3,4-diazol or 1,2,4-triazol, only described for inhibitors of aggrecanase (ADAMTS) or of TACE (Supplementary Fig. S5) [20,21]. Also, 5 compounds also share a 3-pyrazinyl-oxa-2,4-diazol motif and 3 displayed rather hydrophobic groups (either biphenylic, steroidal or long alkyl chain). Out the confirmed hits, 4 compounds (**4-7**) were available in bulk. Potency of **6** and **7**, two compounds that derive from a series we developed as aggrecanase inhibitors [22], was lower than the inhibition of ERAP2 by **4-5** and seemed to plateau at 60% (Supplementary Fig. S6). We thus prioritized the study of the best inhibitors **4** and **5** (Fig. 3).

Fig. 3: Structures and IC_{50} s of hits **4-5**.



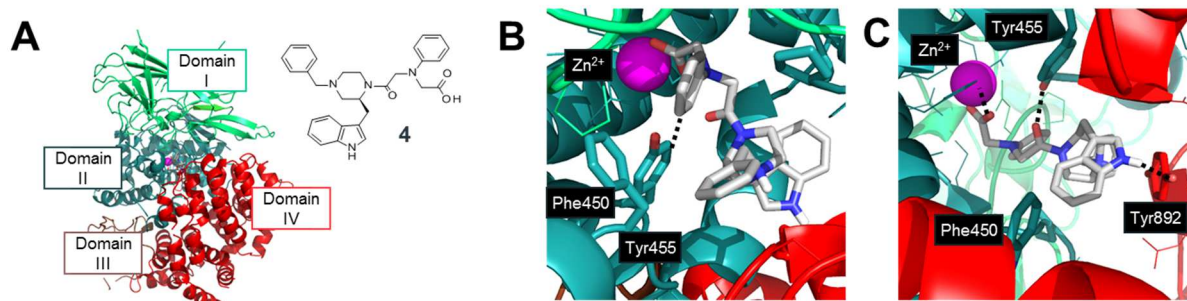
2.3. Binding of hit **4**.

Compound **4** had been previously obtained from the opening of N-substituted iminodiacetic acid anhydrides by a piperazine derivative. Docking of **4** in ERAP2 (Fig. 4) showed that the carboxylic acid interacts with both the Zn^{2+} ion and Histidine 370, and that the carbonyl of the amide function forms a hydrogen bond with Tyrosine 455. Moreover, the phenyl ring of the N-iminodiacetic group stacks between Phe450 (closest distance 3.8 \AA , almost parallel) and the N-benzyl piperazine (closest distance 3.6 \AA , almost T-shaped). Indole group makes a T-shaped interaction with Trp362 (closest distance 3.7 \AA) and is engaged in a hydrogen bond via its NH with the residue Tyrosine 892.

[Tapez ici]

Consistently with the interaction of Tyr892 a selective ERAP2 amino-acid (vs ERAP1), compound **4** is inactive on ERAP1 (%inhibition at 100µM < 10%).

Fig.4: Putative binding mode of **4** in ERAP2^a



^a Docking pose of **4** in PDB 4JBS A: domains of hERAP2; B: Phenyl ring of the iminodiacetic group lies between the benzylpiperazine of **4** and Phe450; C: interactions of **4** with Tyr455 and Tyr892 and Zn²⁺ ion. Oxygens, Nitrogens are in red, blue respectively. Carbons are colored according to domains I, II, III & IV respectively and in gray for **4**. Polar hydrogens of **4** are in white. Zinc ion is represented as a magenta sphere. Polar contacts and interactions are represented as black dashed lines.

Two analogues of **4**, that were already available *in house* (**15-16**), were assayed for their inhibition of ERAP2 (Table 1). Compounds **15** and **16** allowed to study the impact of both the elongation and the shortening of the N-iminodiacetic substituent. Both modifications were deleterious for the activity, suggesting that the phenyl ring at this position is essential for activity and that an N-methyl group is not tolerated at this position.

Table 1: Analogues Hit #4

#cpd	R	ERAP2 IC ₅₀ (µM)
4	Ph-	22±2
15	Ph-CH ₂ -	— ^a
16	CH ₃ -	— ^a

^a (% inh @100µM) below 10%;

2.4. Binding and selectivity of hit **5**.

2.4.1. Docking of **5** in closed hERAP2

Best inhibitor **5** was engaged in a docking study that revealed two putative binding modes (Fig.5 A,B,C and D,E,F respectively).

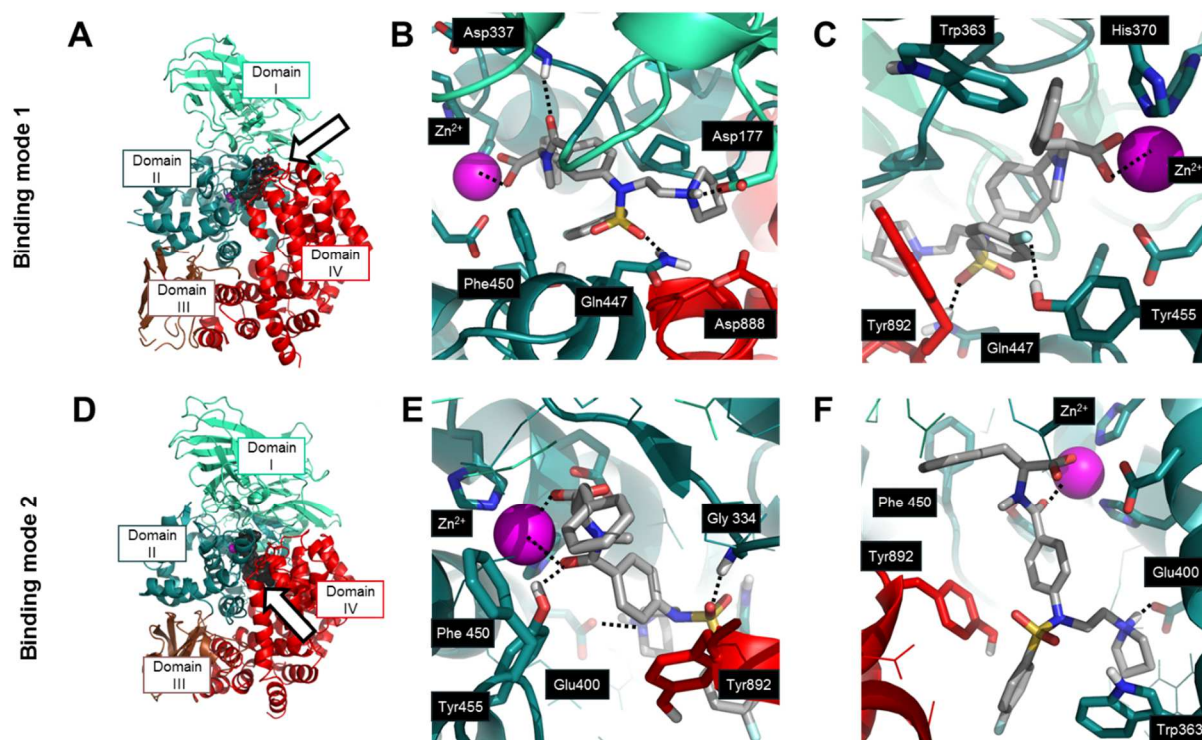
In the first docking pose, the putative binding shows that compound **5** interacts in S1, S1' and S2' pockets of ERAP2 (Fig.5A). The carboxylic acid binds the Zn²⁺ ion. In the S1 pocket, the

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amide group makes a hydrogen bond with the backbone of Asp337, one oxygen of the sulfonamide makes a hydrogen bond with Gln447 and the pyrrolidine ring interacts via an ionic bond with Glu177 (Fig.5B). To our knowledge, there is no reported binding of an inhibitor that goes that deep in S1 pocket. The benzyl ring adjacent to the carboxylic acid, stacks parallel to His370 and orthogonal to Trp363 (T-shaped) in S1' pocket (Fig.5C). The fluoro-phenyl ring interacts with amino-acids at the entrance of the S2' selectivity pocket. It pi-stacks with Tyr892 and makes a hydrogen bond with Tyr 455 (Fig.5C).

In the second possible binding mode, compound **5** interacts deeper in Domain IV (Fig.5D). Both the carboxylic acid and the carbonyl of the amide bind the the Zn^{2+} ion. The carbonyl group forms also a hydrogen bond with the phenol group of Tyr 455 in the S2' selectivity pocket. In S'1 pocket, the tertiary amine (pyrrolidine) and Glu400 are engaged in an ionic bond and the fluoro-phenyl group pi-stacks (T-shaped) with Trp363 (closest distance 2.9 Å). This group also pi-stacks (almost T-shaped) with Tyr 892 from the S2' selectivity pocket. In this pose, fewer interactions are seen with the S1 pocket. The benzyl group engages in T-shaped stacking with Phe450 (closest distance 3.4 Å) and the oxygen of the sulfonamide forms hydrogen bond with Gly 334 backbone.

Fig.5: Docking poses of **5** in ERAP2^a



^a Two docking poses of **5** (A,B and C) and (D, E and F) in PDB 5J6S.(A and D) general ERAP2 views showing the two different putative binding modes of **5**, either upwards, towards Domain I or downwards, towards Domain IV. (B,C) and (D,E) closer views of the first or second binding modes respectively.**5** could

[Tapez ici]

interact with new residues like Glu177 or Glu400 through its pyrrolidine substituent. Oxygens, Nitrogens are in red, blue respectively, Carbons are colored according to domains and in gray for **5**. Polar hydrogens of **5** are in white. Zinc ion is represented as a magenta sphere. Polar contacts and interactions are represented as black dashed lines.

2.4.2. Selectivity of **5**

So far, published inhibitors of ERAP2 retain activity on the homologous ERAP1 and/or IRAP. IRAP (Insulin-regulated aminopeptidase) is another protease from the M1-family to which ERAP enzymes belong. It is also implicated in antigen presentation, in particular in the processing of precursors derived of endocytosed proteins in dendritic cells for cross-presentation. All three enzymes share 50% homology in sequence and display very conservative catalytic sites. Several ERAP1 selective inhibitors [23] or IRAP selective inhibitors [24] have been disclosed. As low selectivity of ligands between the three enzymes, could preclude the understanding their respective implication in pathologies and the preclinical development compounds, we assessed selectivity of hit **5** towards ERAP1 and IRAP (Table 2). Compound **5** is selective of ERAP2 other these two other enzymes. It is indeed completely inactive on ERAP1 and shows a 1-log selectivity towards ERAP1. **5** is thus a good starting point for pharmacomodulation.

Table 2: Selectivity of **5**

#cpd	ERAP2 IC ₅₀ (μM)	ERAP1 IC ₅₀ (μM) ^a	IRAP IC ₅₀ (μM) ^a
5	9.7±5.0	— (10)	≈100 (49)

^a in parenthesis % inhibition @ 100μM;

2.5. Design and synthesis of analogues of **5**

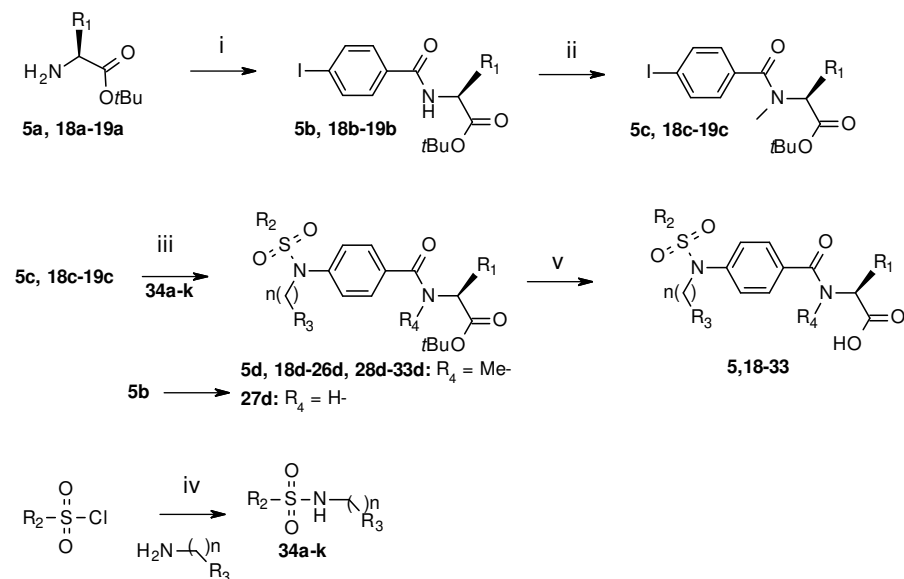
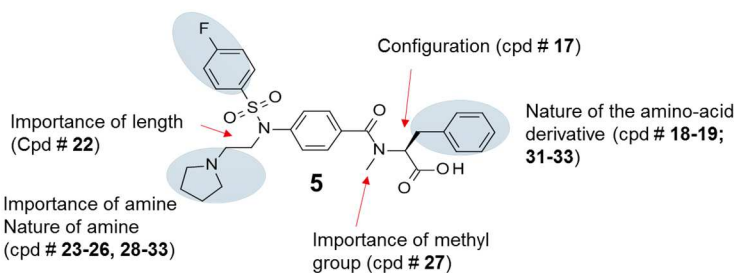
2.5.1. Design of analogues of **5** to explore binding

Analogues of **5**, **17-33** (Fig. 6) were designed to validate interactions identified in docking and key structural elements like configuration (**17**), nature of amino-acid (**18-19,31-33**), importance of tertiary amine and linker length (**22-26, 28-33**), importance of the methylation of amide (**27**), importance of aryl group (**20-21**).

Fig.6: Design of analogues **17** to **33**.

[Tapez ici]

Importance aryl group
Importance of Fluorine
(cpd # 20-21)



Intermediates:		Final cpds:					
R ₁	R ₂	R ₁	R ₂	R ₃	n		
R ₄ = Me-							
5a-c	benzyl-	5	5d	4-fluorophenyl-	2-pyrrolidin-1-ylethyl-	2	
18a-c	isobutyl-	18	18d	4-fluorophenyl-	2-pyrrolidin-1-ylethyl-	2	
19a-c	phenethyl-	19	19d	4-fluorophenyl-	2-pyrrolidin-1-ylethyl-	2	
		20	20d	benzyl-	Me-	2-pyrrolidin-1-ylethyl-	2
34a	Ph-(CH ₂) ₂ -	21	21d	benzyl-	Ph-	2-pyrrolidin-1-ylethyl-	2
34b	Ph-(CH ₂) ₂ -	22	22d	benzyl-	4-fluorophenyl-	2-pyrrolidin-1-ylethyl-	3
34c	Ph-(CH ₂) ₂ -	23	23d	benzyl-	4-fluorophenyl-	2-(diethylamino)ethyl-	2
34d	2-pyrrolidin-1-ylethyl-	24	24d	benzyl-	4-fluorophenyl-	2-dimethylamino)ethyl-	2
34e	2-pyrrolidin-1-ylethyl-	25	25d	benzyl-	4-fluorophenyl-	2-(piperidin-1-yl)ethyl-	2
34f	2-pyrrolidin-1-ylethyl-	26	26d	benzyl-	4-fluorophenyl-	2-morpholinoethyl-	2
34g	2-pyrrolidin-1-ylethyl-	28	28d	benzyl-	Ph-	Ph-(CH ₂) ₂ -	2
34h	2-(diethylamino)ethyl-	31	31d	isobutyl-	Ph-	Ph-(CH ₂) ₂ -	2
34i	2-dimethylamino)ethyl-	29	29d	benzyl-	4-fluorophenyl-	Ph-(CH ₂) ₂ -	2
34j	2-(piperidin-1-yl)ethyl-	32	32d	isobutyl-	4-fluorophenyl-	Ph-(CH ₂) ₂ -	2
34k	2-morpholinoethyl-	30	30d	benzyl-	Me-	Ph-(CH ₂) ₂ -	2
		33	33d	isobutyl-	Me-	Ph-(CH ₂) ₂ -	2
R ₄ = H-							
		27	27d	benzyl-	4-fluorophenyl-	2-pyrrolidin-1-ylethyl-	2

Scheme 1: Synthesis of analogues 5,18-33.

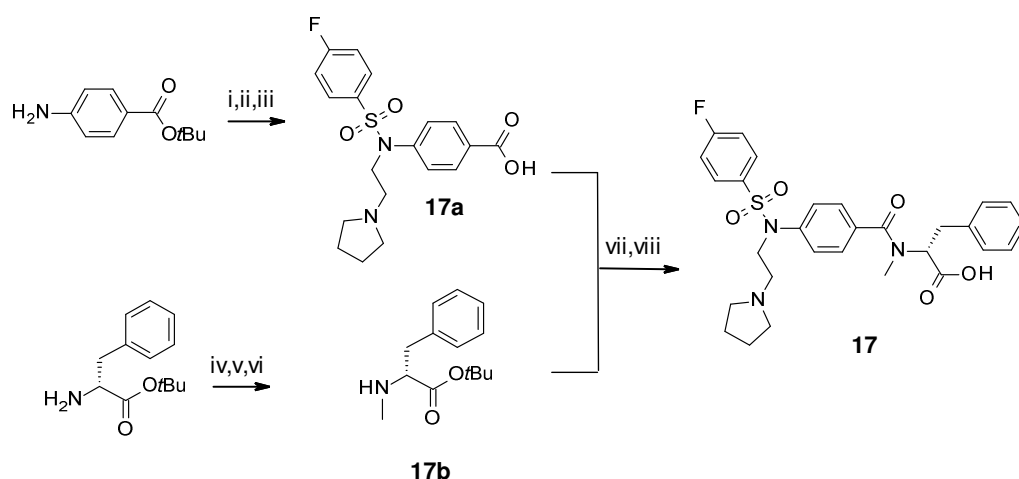
Reactions and conditions: i) 4-iodobenzoyl chloride (1 eq.), NEt₃ (4 eq.), *t*-butyl ester of amino acid (1 eq.), CH₂Cl₂, r.t.; 4 h ii) NaH (1.3 eq.)/anh.THF; 0 °C, 1h, then MeI (2.0 eq.), r.t., overnight. iii) Cul (0.1 eq.), K₃PO₄ (2 eq.), argon, then sulfonamide (1.1 eq.)/anh. 1,4-dioxane, *trans*-1,2-diaminocyclohexane (0.2 eq.), 110 °C, 36 h, or Cul (0.1 eq.), K₂CO₃ (2.5 eq.), argon, then sulfonamide (1.1 eq.)/anh. ACN, N,N'-dimethylethane-1,2-diamine (0.7 eq.), 80 °C, 48 h iv) sulfonyl chloride (1 eq.), NEt₃ (1.2 eq.) in CH₂Cl₂, 0 °C, then amine (1.1 eq.), 0 °C to r.t., 12h. v) *t*-butyl ester (1.0 eq.)/CH₂Cl₂, iPr₃SiH (0.1 mL), TFA, r.t, 1 h, or 4N HCl/1,4-dioxane, CH₂Cl₂, r.t.,24 h.

2.5.2.Synthesis of analogues of 5

Compound 5 and its analogues 18-33 were synthesized in 4 steps from the corresponding amino-acid via a sequence including an Ullman reaction (Scheme 1). Tertbutylaminoester was reacted with 4-

[Tapez ici]

iodobenzoyl chloride to give the corresponding amide. Then the amide was methylated with iodomethane in the presence of sodium hydride. The iodinated derivatives were then reacted in a copper-catalyzed Ullman reaction with sulfonamides (**34a-k**) previously synthesized from the corresponding amine and sulfonylchloride. Iodinated derivatives and sulfonamides reacted in the presence of CuI, K₂CO₃ and *trans*-N,N'-dimethylcyclohexane-1,2-diamine or N,N'-dimethylethane-1,2-diamine under inert atmosphere, to give the corresponding arylated sulfonamides [25]. The *tert*-butyl protecting group was removed under acidic conditions (TFA or HCl 4M). Alternatively, we developed a second synthetic route (Scheme 2), that we used to synthesize analogue **17**. First, sulfonylchloride was substituted by *tert*-butyl 4-aminobenzoate in pyridine. Then the resulting sulfonamide was alkylated using 1-(2-chloroethyl)pyrrolidine hydrochloride in DMF in the presence of K₂CO₃, to introduce the protonated side chain, followed by deprotection of the *tert*-butyl group in HCl/dioxane, to give carboxylic acid **17a**. To synthesize amine **17b**, 2-nitrobenzenesulfonyl chloride was first reacted with *D*-phenylalanine *tert*-butyl ester to give *tert*-butyl (2*S*)-2-[(2-nitrophenyl)sulfonylamino]-3-phenyl-propanoate. The latter was alkylated using iodomethane. Then, the (2-nitrophenyl)sulfonyl protecting group was removed in DMF, using mercaptoacetic acid (8 eq.), Cs₂CO₃ (2 eq.) and LiOH·H₂O (7 eq.). N-methylated derivative **17b** reacted with **17a** to form the amide bond and deprotection under acidic conditions (HCl 4M) gave analogue **17**.



Scheme 2: Synthesis of **17**.

Reactions and conditions: i) *p*-fluoro-sulfonyl chloride, pyridine, CH₂Cl₂, 70 °C, 3h ii) 1-(2-chloroethyl)pyrrolidine, K₂CO₃ (3 eq.), DMF, r.t., overnight; iii) 4N HCl/dioxane, CH₂Cl₂, r.t., overnight; iv) 2-nitrobenzenesulfonyl chloride; K₂CO₃ (2 eq.), DMF, 0 °C -> r.t., overnight; v) CH₃I (4 eq.), K₂CO₃ (2 eq.), DMF, 0 °C -> r.t., overnight; vi) mercaptoacetic acid (4 eq.), Cs₂CO₃ (1 eq.), LiOH·H₂O (1 eq.), DMF, 60 °C, overnight; vii) HOBT (0.55 eq.), HBTU (0.16 eq.), TEA (2.2 eq.), DMF, r.t., overnight; viii) 4N HCl/1,4-dioxane, CH₂Cl₂, r.t., 24 h.

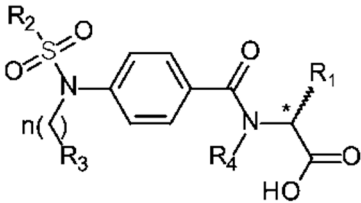
2.6. Structure-activity relationships of inhibitors, analogues of **5**

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We evaluated structural modifications on **5** while keeping the central scaffold (sulfonamide-aryl-amide) (compounds **17-27**, Table 3). Hit **5** derived from *S*-phenylalanine. Changing the conformation of the C α (*R* isomer **17**) resulted in a complete loss of activity. Replacement of the R₁ benzyl group of **5** by an *iso*-butyl (derivative of leucine **18**) or by the phenethyl homolog group (**19**) was deleterious for activity, suggesting that larger groups and/or non-aromatic groups are not tolerated at this position. The removal of the phenyl ring on the sulfonamide group resulted also in a decrease in activity (**20**), pointing out a need for an aromatic (or large) group at this position. Interestingly, the fluorine atom in *para* position on this phenyl ring seems essential for activity as its removal led to a loss of activity (**5** vs **21**).

Elongation of the chain (n=3) of the R₃ substituent leads to a 5-fold decrease in activity (**22**). The introduced flexibility may compensate partially the increasing size of the linker. Several tertiary amines either cyclic or acyclic (**23-26**) have been explored to study the impact of the replacement of the N-substituted pyrrolidine ring, while keeping a charge. All analogues were less active than reference **5**. Finally, the removal of the methyl group on the amide (**27**) led to a 5 fold decrease in activity, suggesting that a hydrogen-bond donor at this position is not tolerated. All these activities are in line with the docking results where multiple stackings were found between aryl groups and aromatic amino-acids, interactions between hydrogen bonds acceptors CO of the amide or SO₂ of the sulfonamide, and ERAP2. The results obtained for compounds **23-26** is further discussed below.

Table 3: Analogues of hit **5**



#cpd	C*	R ₁ -	R ₂ -	n	R ₃ -	R ₄ -	IC ₅₀ μM (%inh) ^a
5	S	benzyl-	4-F-C ₆ H ₄ -	2	<i>N</i> -pyrrolidinyl-	Me-	9.7±5.0
17	R	benzyl-	4-F-C ₆ H ₄ -	2	<i>N</i> -pyrrolidinyl-	Me-	(30)
18	S	<i>iso</i> -butyl-	4-F-C ₆ H ₄ -	2	<i>N</i> -pyrrolidinyl-	Me-	(14)
19	S	phenethyl-	4-F-C ₆ H ₄ -	2	<i>N</i> -pyrrolidinyl-	Me-	(14)
20	S	benzyl-	CH ₃ -	2	<i>N</i> -pyrrolidinyl-	Me-	(24)
21	S	benzyl-	C ₆ H ₅ -	2	<i>N</i> -pyrrolidinyl-	Me-	— ^c
22	S	benzyl-	4-F-C ₆ H ₄ -	3	<i>N</i> -pyrrolidinyl-	Me-	≈100 ^b
23	S	benzyl-	4-F-C ₆ H ₄ -	2	<i>N</i> -diethylamino-	Me-	— ^c
24	S	benzyl-	4-F-C ₆ H ₄ -	2	<i>N</i> -dimethylamino-	Me-	— ^c
25	S	benzyl-	4-F-C ₆ H ₄ -	2	<i>N</i> -piperidinyl-	Me-	— ^c
26	S	benzyl-	4-F-C ₆ H ₄ -	2	<i>N</i> -morpholinyl-	Me-	(30)
27	S	benzyl-	4-F-C ₆ H ₄ -	2	<i>N</i> -pyrrolidinyl-	H-	≈100 ^b

^a: % inh given @ 100μM; ^b % inhibition @ 100 μM = 54-55%; ^c % inhibition below 5% @ 100μM.

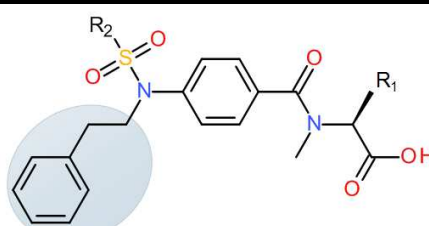
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2.7. Discovery of hERAP2 activators, analogues of **5**

2.7.1. SAR of Activators of ERAP2-mediated hydrolysis of small peptidic substrates.

As dockings studies and SAR described in Table 3 emphasize the importance of the basic side chain in R₃ position, we decided to challenge this hypothesis by synthesizing analogues bearing a phenyl ring, instead of the pyrrolidine ring, at this position (Compounds **28-33**, Table 4). Surprisingly, for analogues of **5** bearing a phenethyl group as the N-substituent of the sulfamide, activity shifted from inhibition to activation in 143% to 184%. If R₁ derived of phenylalanine, activation of ERAP2 was slightly greater than the isoleucine analogues (**28-29** vs **31-32**), in striking difference with inhibitors series where the replacement of the phenylalanine in R₁ by isoleucine led to complete loss of potency (**5** vs **18** in Table 3). The R₂ position impacts greatly the activator behavior as replacement of the aryl sulfonamide by a methyl-sulfonamide group provides completely inactive compounds, **30** and **33**, regardless of the R₁ group (neither activators, nor inhibitors). Whereas for inhibitors the fluorine atom seemed important (probably to enhance pi-stacking), here, phenyl and fluoro-phenyl analogues are almost equivalent (**28,31** vs **29, 32** respectively). Analogue **29** (fold=1.84 at 100μM) is the most active in the series, showing a clear dose dependent activation of ERAP2 towards the hydrolysis of R-AMC substrate. All compounds are selective of ERAP2 over ERAP1 (for example for **28-29**: % inhibition @ 100μM < 20% on ERAP1 and ≈ 50% on IRAP).

Table 4: Phenethyl derivatives **28-33**



#cpd	R ₁ -	R ₂ -	Activation fold @ 100μM ^a
28	benzyl-	C ₆ H ₅ -	1.76±0.02
29	benzyl-	4-F-C ₆ H ₄ -	1.84±0.06
30	benzyl-	CH ₃ -	^b
31	<i>iso</i> -butyl-	C ₆ H ₅ -	1.43±0.12
32	<i>iso</i> -butyl-	4-F-C ₆ H ₄ -	1.46±0.04
33	<i>iso</i> -butyl-	CH ₃ -	^b

^a relative to basal hERAP2 activity ; ^b no activation, no inhibition

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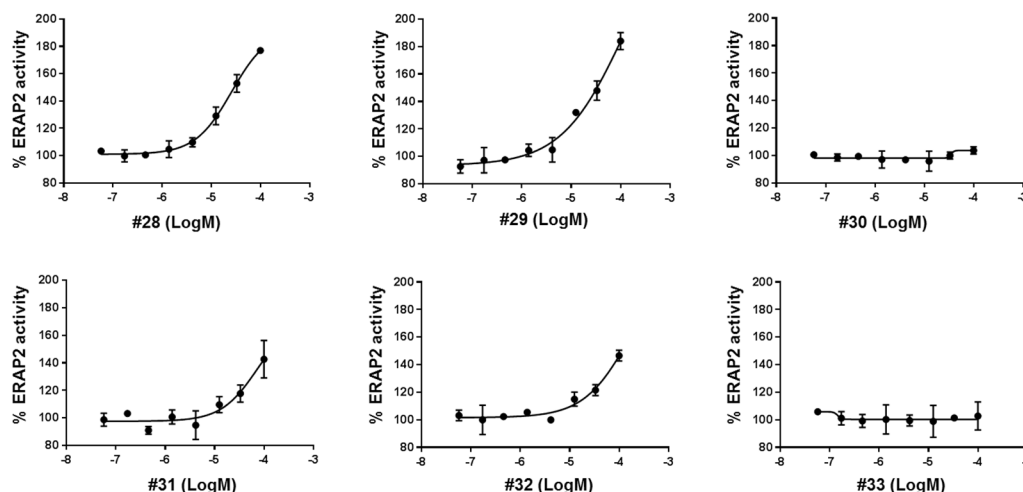
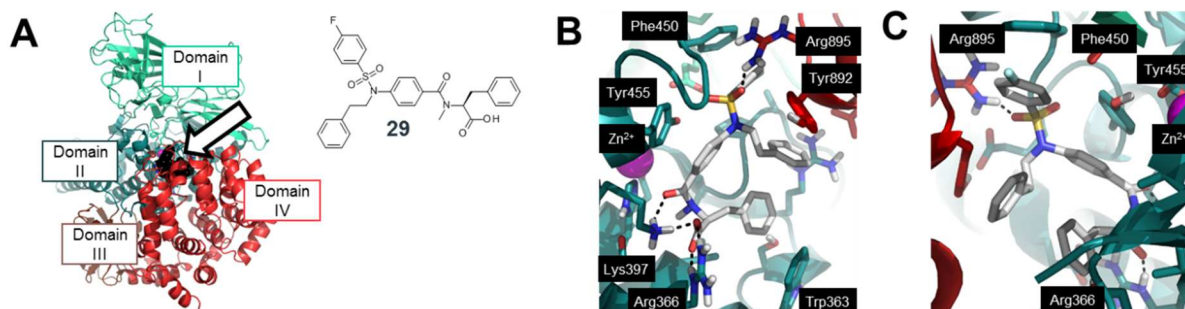


Fig.6: DRCs of the 6 phenethyl derivatives (**28-33**).^a

^a Compounds **28-29** and **31-32** behave as activators of Arg-AMC hydrolysis by hERAP2.

2.7.2. Docking study of **29** in ERAP2.

Fig.7: Putative binding mode of **29** in ERAP2^a



^a Docking pose of **29** in PDB 5J6S. A: binding pocket of **29** at the junction of Domain II and IV of ERAP2; B and C : **29** lies in the catalytic pocket at distance from the Zinc ion. Oxygens, Nitrogens are in red, blue respectively, Carbons are colored according to domains I, II, III & IV respectively and in gray for **29**. Polar hydrogens are in white. Zinc ion is represented as a magenta sphere. Polar contacts and interactions are represented as black dashed lines.

We hypothesized that phenethyl analogues of **5** might bind to a different pocket in ERAP2 due to the lack of the pyrrolidine ring. We thus docked compound **29** in ERAP2 (Fig. 7). Interestingly, we found that analogue **29** lies nonetheless in the catalytic site (Fig. 7A) but does not interact with the Zn^{2+} ion (Fig. 7B,7C). The carboxylic acid function of **29** makes polar contacts with Arg366 and Lys397. Furthermore, amide and sulfonamide groups are engaged in hydrogen bonds with Lys397 and Arg895 respectively. **29** adopts a U-shape allowing multiple π -stackings with ERAP2 residues involving both ERAP2 residues and intramolecular phenyl rings (Fig. 7B,7C). More specifically, the aryl ring of the phenethyl moiety, specific of these analogues, stacks with Tyr892 (closest distance 3.2 Å) and benzyl group of **29** (T-shaped 3.5 Å) (Fig. 7B). Also, Tyr455, the para-disubstituted benzene central core, the

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benzyl group and Trp363 stack together (distances between 3.1-3.8 Å). Finally, fluoro-phenyl group stacks with Phe 455 (Fig. 7C: closest distance 3.2 Å)

2.8. Hydrolysis KSIINFEKL nonapeptide precursor peptide

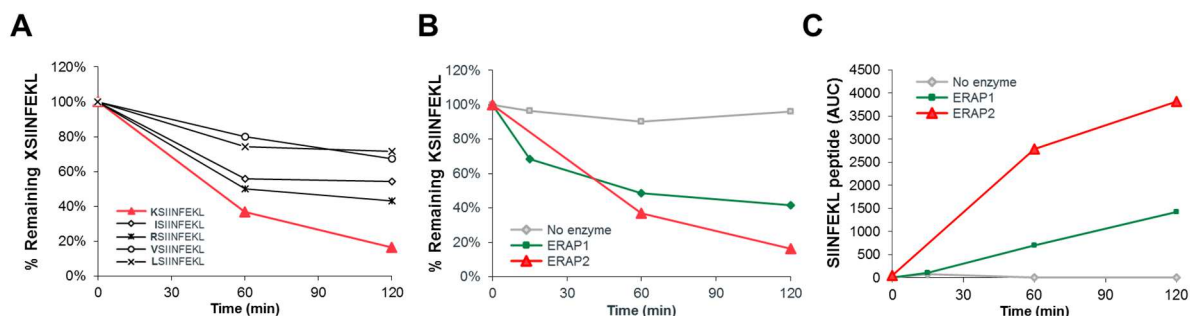


Fig.8: Selection of SIINFEKL-derived peptide for hydrolysis by ERAP2 preferably. ^a

^a A: Hydrolysis of 9-mer or 10-mer peptides derived of SIINFEKL by ERAP2. KSIINFEKL is the best substrate. B: KSIINFEKL is a better substrate for ERAP2 than for ERAP1. C: Hydrolysis of KSIINFEKL by ERAP enzymes produces epitope SIINFEKL. Peptides were titrated by LCMS-MS after 2 hours. Data are mean of 2 experiments.

To explore the inhibition or activation profile of analogues of **5** towards the hydrolysis by ERAP2 of longer, more relevant peptides, we looked at nonapeptides meant to produce SIINFEKL epitope. First, we identified by LCMS-MS, KSIINFEKL and RSIINFEKL as best potential substrates for hydrolysis by ERAP2 as expected in the light of preferences for basic side chain in S1 described for this enzyme. In particular KSIINFEKL was readily hydrolyzed by ERAP2 (Fig.8A), but not as rapidly hydrolyzed by ERAP1 (Fig.8B). We checked that the hydrolysis of KSIINFEKL by ERAP enzymes was correlated with the production of the corresponding epitope SIINFEKL (Fig. 8C). Next we evaluated the impact of the presence of inhibitor **5** or activators **28** and **29** on the hydrolysis of KSIINFEKL by ERAP2 and the production of SIINFEKL.

Interestingly all three compounds are inhibitors of the hydrolysis of nonapeptide by ERAP2 in a dose-dependent manner (Fig.9). **5** ($IC_{50} = 98 \mu M$) and **28** ($IC_{50} = 89 \mu M$) are the most potent inhibitors. We checked that the decrease of KSIINFEKL trimming by ERAP2 in the presence of inhibitors was correlated with the decrease of SIINFEKL production (Supplementary Fig. S7).

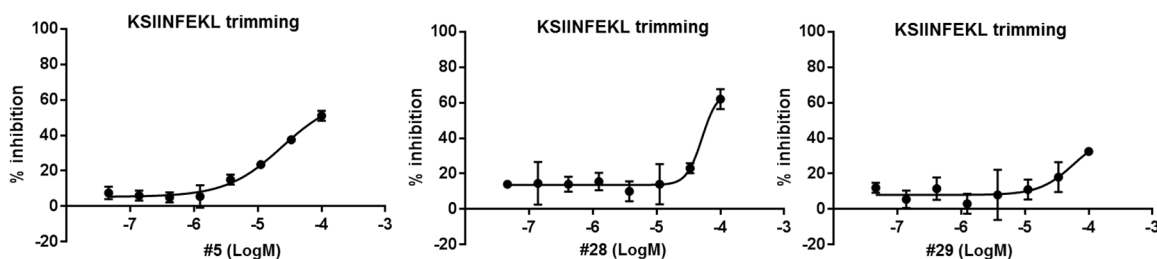


Fig.9: Dose dependent inhibition of ERAP2-mediated RSIINFEKL hydrolysis by **5**, **28** & **29**. ^a

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^a Compounds **5**, **28-29** inhibit ERAP-2 catalyzed hydrolysis of the N-terminal arginine from inhibit processing of a common 9mer peptide KSIINFEKL precursor of the mature antigenic epitope from chicken ovalbumin SIINFEKL, as measured by mass-spectrometry after 60 minutes. Data are mean of two experiments

2.9. Discussion

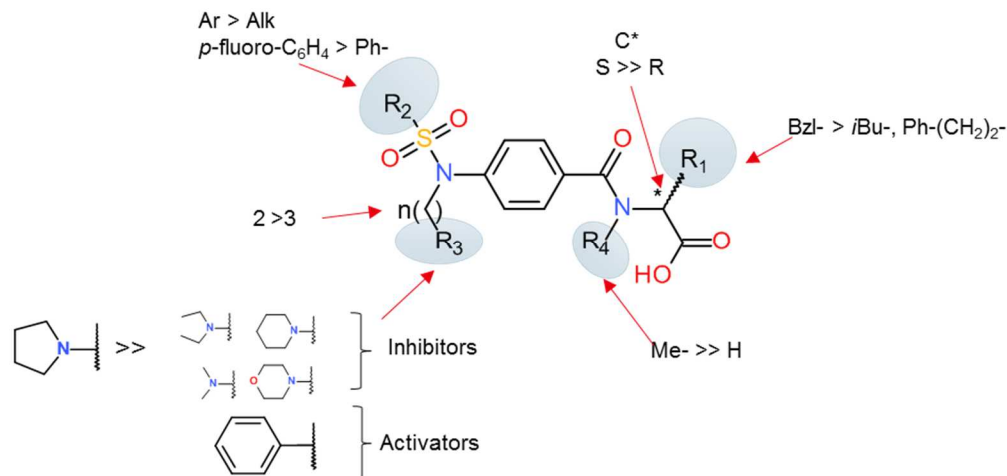


Fig.10: SAR in the series of **5**, towards hydrolysis of small substrates by ERAP2.

Inhibitors **4** and **5** were discovered during an HTS campaign, and were active in the micromolar range on the hydrolysis of short model substrates (Arg-AMC) by ERAP2. Structure-activity relationships, in particular for **5**, are depicted in Figure 10.

Docking studies showed that they both interact with the Zn²⁺ within the catalytic site of the enzyme thanks to a carboxylic acid moiety. Key residues of ERAP2 have previously been highlighted for their role in the catalytic mechanism and/or the binding of inhibitors. For example, Tyr455 next to the catalytic center (interface between S1 and S'2) reorient during ligand binding, stabilizes the transition state during catalysis and is implicated in the open-closed conformation based on results obtained on ERAP1 [26,27]. Also, Phe450 is a key residue of the S1 pocket, whose ERAP1 counterpart (Phe433) has been shown to be disordered or dislocated in the open state of the enzyme [28]. Both hits **4** and **5** interact with either pi-stacking or hydrogen-bonds with these two residues. Trp363 (S'1) and Tyr892 (S'2) are amino-acids selective of ERAP2 vs ERAP1. The interaction of **4** with Tyr892 and of **5** with both residues may explain their selectivity towards ERAP2.

In **5**, the N-substituted pyrrolidine is essential for inhibition for both steric and protonation state reasons. Indeed, its replacement by other tertiary amines either cyclic or acyclic (**23-26**) led to a complete loss of activity, consistently with the pKa reduction (from pKa=9.85 to 5.9 ≤ pKa ≤ 9.6 for the

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analogues). Docking revealed that this group is involved in ionic interactions either with Glu177 or Glu400 in the S1 pocket.

This is the first time to our knowledge that an inhibitor binds either of these two residues. Interestingly, whereas Glu400 is conserved in ERAP1 (Glu383), Glu177, a residue of the capping region of S1, is specific of ERAP2 and replaced by a histidine in ERAP1 (His160) or a tyrosine in IRAP (Tyr272). Furthermore this residue has been shown to be implicated in specific interactions with domain IV (Asp188) in the allele K192N (Fig. 4B), supposedly affecting the open-close conformational equilibrium of the enzyme and thus its catalytic activity [29]. This opens new perspectives in the exploration of the S1 pocket.

Earlier studies have shown that ERAP1 has an original trimming mechanism ending up with a specificity to hydrolyze 9-16-residue precursors, thanks to the presence of an allosteric pocket that recognize specific C-term amino-acids of substrates (pocket defined by Tyr684, Lys685, and Arg807). It has also been shown that this mechanism explains the capacity of small peptides to activate the enzyme [26,30]. Recently, an activator/inhibitor of ERAP1 was discovered by screening and found to bind a pocket (K551, T914) distant from the catalytic site [31]. Similarly, a small compound was discovered as activator of the hydrolysis of a small substrate of ERAP1 (Leu-AMC), but inhibitor of the degradation of larger substrates [32]. Docking studies suggested that the latter compound may bind a regulatory site. This binding mode explains why the hydrolysis of short peptides that do not engage the regulatory site is promoted; while the hydrolysis of longer peptides, whose C-terminus normally occupies the same allosteric site, is blocked, due to their binding being hindered by the compound.

The binding of peptides to this allosteric regulatory site promotes the flipping of domains I and IV required for the conformational change of ERAP1 from open form to closed form, as well as for the active-site reorganization [27].

This mechanism is thought to be unique to ERAP1 in comparison with ERAP2 and IRAP. For example, it has been shown that ERAP2 is not activated by peptides and prefers to trim shorter peptides [26]. No allosteric pocket, neither activators have been disclosed so far for ERAP2.

In the course of the exploration of structure-activity relationships around hit **5**, we discovered a sub-series, namely **28-33**, where the pyrrolidine ring of **5** has been replaced by a phenyl group. These analogues are the first activators of small substrates hydrolysis by ERAP2. Surprisingly, docking study did not reveal binding of **29** to a remote site in ERAP2. Indeed, analogue **29** does bind the active site

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but away from the Zn²⁺ catalytic ion and makes no interactions with Domain I at all. In contrast with inhibitors like **4** or **5**, which bind the Zn²⁺ catalytic ion and slip between this ion and the catalytically important residue Glu200, compound **29** leaves both the Zn²⁺ and Glu200 accessible and seems to shape the catalytic site, efficiently for adequate binding of small substrates like Arg-AMC (Fig. 11). Still, as it is located in the active site, longer peptides cannot bind anymore and thus the activity of **29** shifts from activation of small substrates hydrolysis to inhibition of larger substrate hydrolysis.

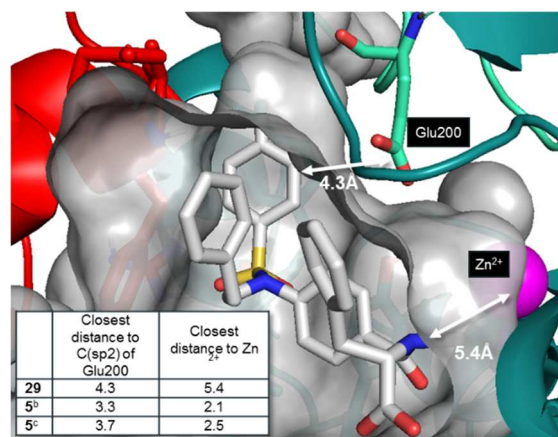


Fig. 11: Zoom of the catalytic chamber in ERAP2 in the presence of activator/inhibitor **29**.^a

^a Docking pose of **29** in PDB 5J6S. Oxygens, Nitrogens are in red, blue respectively, Carbons are colored according to domains I, II, III & IV respectively and in gray for **29**. Polar hydrogens are in white. Zn²⁺-ion is represented as a magenta sphere. Distances are in Å

3. Conclusion.

Thanks to screening, we identified 11 drug-like inhibitors of ERAP2, two of which, compound **4** and **5**, were further characterized. Both these compounds are selective of ERAP2 over the closely related enzymes ERAP1 and IRAP. Several analogues were synthesized and Structure-Activity Relationships confirmed docking studies that showed they inhibit competitively ERAP2 in the catalytic site. Interestingly, **5** binds so far untapped amino-acids in S1 pocket and may be useful as a starting point for future optimizations. In the course of analogue design, we identified the first activators of ERAP2. These act as activators of Arg-AMC hydrolysis, but inhibit the processing of nonapeptides. Based on docking, these activator/inhibitor compounds appear to bind in the catalytic site, but away from the catalytic zinc and Glu200, a completely new binding mode for ERAP2 modulators. Screening using small substrates allows thus to identify ligands with partial occupancy of the binding pocket and which can be used to study the pocket bit by bit and be new starting point for optimization. Still, different substrates including more physiological peptides are necessary to select molecules that will be likely to have *in vivo* effect consistent with their *in vitro* effect.

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4. Experimental section

4.1.1. *In vitro* ERAP2 activity assay.

ERAP2 was prepared as previously described [13]. The enzymatic activity of ERAP2 was assayed using R-AMC (L-Arginine-7-amido-4-methylcoumarin hydrochloride, Sigma). Hepes at 50 mM with 100 mM NaCl at pH 7 was used as buffer. Briefly, 60 nL of test compounds were added in 384-wells plates (dark, non-binding surface) by acoustic dispensing with Echo (Labcyte) and pre-incubated 30 minutes at ambient temperature with 10 μ L of ERAP2 1 μ g/mL or vehicle. The reaction was then started with the addition of 10 μ L of substrate at 10 μ M. The final concentration of ERAP2, substrate and DMSO was 0.5 μ g/mL, 5 μ M and 0.4% respectively. For the kinetic readout a Victor 3V (Perkin-Elmer) was used with excitation at 380 nm and emission at 450 nm. The fluorescence was measured each 3 minutes during one hour.

The Z and Z' factors were calculated according to J.-H. Zhang, T.D.Y. Chung, K.R. Oldenburg, A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays, J. Biomol. Screen., 4 (1999) 67-73. Data analysis was performed using Xlfit® v 5.0 or GraphPad Prism® v 4.0.

4.1.2. *Dose-Response Curves.*

Percentages of inhibition or activation at different concentrations were obtained as for screening. All measurements were carried out as 8-point dose response curves and reported as the average of at least three independent measurements. Bestatin was used as a reference inhibitor (100% inhibition at 2 mM). Data analysis was performed using Xlfit® v 5.0 or GraphPad Prism® v 4.0. Nonlinear curve fitting and statistical analysis was done using built-in functions.

4.1.3. *Selectivity*

In vitro ERAP1 and IRAP activity assays were performed as for ERAP2 using L-AMC (L-Leucine 7-Amido-4-MethylCoumarin) from Sigma, as the substrate, recombinant human ERAP1 (PILS/ARTS1 from R&D Systems, ref 2334-ZN-010) at 0.8 μ g/mL final concentration or recombinant human IRAP (prepared as previously described [17]) at 0.2 μ g/mL final concentration respectively.

4.1.4. *Hydrolysis of nonamer-peptides*

Enzymatic reactions were performed as previously described using X-SIINFELK peptides (from Proteogenix, Schiltigheim, FR). The enzymatic reactions were stopped at the desired time-point by dilution using iced acetonitrile (x100 dilution), before injection in LC-MS/MS to measure AUC. LC-MS/MS analysis were performed on an UPLC system Acquity I Class (Waters®), combined with a triple quadrupole mass spectrometer Xevo TQD (Waters®). The column was an Acquity BEH C18 50*2.1 mm, 1.7 μ m column (Waters®) and the following mobile phases were used: 5mM ammonium formate pH 3.75 buffer for solvent (A) and 5 mM ammonium formate pH 3.75 in acetonitrile for solvent (B). At a flow rate of 600 μ L/min, the analytical method starts at 98% (A) for 10s, then the percentage of B gradually increases at 98% till 2 minutes, hold at 98% (B) for 30s before returning to the initial

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conditions, hold 1.5 minutes. The injection volume was 1 μ L. MS analyses were performed under MRM detection using the parameters optimized for each peptide (capillary voltage, product ions, collision energy, desolvation temperature) (Supplementary Table S1). The control of the equipment as well as the reprocessing of the analyses were carried out using MassLynx software (Waters®). For XSIINFEKL, 100% corresponds to the AUC of the peptide at t=0 without enzyme. Dose-response curves with compounds were performed at t= 60 min.

4.1.5. Docking and simulation calculations.

pKa were calculated using PipelinePilot® 8.5 from Dassault systems.

Modeling and simulations were performed using non-aged X-ray crystal structure of ERAP2 PDB 5J6S. The protein was protonated then charged using AM1-BCC in Chimera 1.13 [33]. Then, docking calculations were performed using rDock 2013.1 [34]. A zinc chelation pharmacophore sphere located on the crystal ligand's oxygens positions has been added with a binding energy coefficient of 100 to ensure zinc chelation. Fifty poses have been generated for each molecules. Each poses have been manually inspected. Then, shortlisted poses have been refined using 2018.0101 (Chemical Computing Group, Inc.) to highlight protein-ligand interactions. The refinement process involved a re-protonation of the system at 310K, pH7. The minimization was performed using the Forcefield optimization with Amber14. When water was kept in the pocket, a first run were done with rigid water, then a second run were done with unfrozen water.

The structures were rendered in PyMOL (Delano, W. L. The PyMOL Molecular Graphics System. DeLano Scientific LLC: San Carlos, CA, 2002).

4.1.6. Chemistry.

4.1.6.1. General information.

Solvents for synthesis, analysis and purification were purchased as analytical grade from commercial suppliers and used directly without further purification. Chemical reagents were purchased as reagent grade and used without further purification.

LC-MS Waters system was equipped with a 2747 sample manager, a 2695 separations module, a 2996 photodiode array detector (200-400 nm) and a Micromass ZQ2000 detector (scan 100-800). XBridge C18 column (50 mm x 4.6 mm, 3.5 μ m, Waters) was used. The injection volume was 20 μ L. A mixture of water and acetonitrile was used as mobile phase in gradient-elution. The pH of the mobile phase was adjusted with HCOOH and NH₄OH to form a buffer solution at pH 3.8. The analysis time was 5 min (at a flow rate at 2 mL/min), 10 min (at a flow rate at 1 mL/min) or 30 min (at a flow rate at 1 mL/min). Purity (%) was determined by reversed phase HPLC, using UV detection (215 nm), and all isolated compounds showed purity greater than 95%.

HRMS analysis was performed on a LC-MS system equipped with a LCT Premier XE mass spectrometer (Waters), using a XBridge C18 column (50 mm x 4.6 mm, 3.5 μ m, Waters). A gradient starting from 98% H₂O 5 mM Ammonium Formate pH 3.8 and reaching 100% CH₃CN 5 mM Ammonium Formate pH 3.8 within 3 min at a flow rate of 1 mL/min was used.

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NMR spectra were recorded on a Bruker DRX-300 spectrometer. The results were calibrated to signals from the solvent as an internal reference [e.g. 2.50 (residual DMSO-d₆) and 39.52 (DMSO-d₆) ppm for ¹H and ¹³C NMR spectra respectively]. Chemical shifts (δ) are in parts per million (ppm) downfield from tetramethylsilane (TMS). The assignments were made using one-dimensional (1D) ¹H and ¹³C spectra and two-dimensional (2D) HSQC-DEPT, COSY and HMBC spectra. NMR coupling constants (J) are reported in Hertz (Hz), and splitting patterns are indicated as follows: s for singlet, brs for broad singlet, d for doublet, t for triplet, q for quartet, quin for quintet, dd for doublet of doublet, ddd for doublet of doublet of doublet, dt for doublet of triplet, qd for quartet of doublet, m for multiplet, δ for chemical shift, J for coupling constant.

Flash chromatography was performed using a Puriflash®430 with silica columns. UV and ELSD detection were used to collect the desired product.

Reverse flash chromatography was performed using a CombiFlash® C₁₈ Rf200 with C₁₈ silica gel cartridges. UV detection (215 and 254 nm) was used to collect the desired product.

Preparative HPLC was performed using a Varian PRoStar system with an OmniSphere 10 μ m column C₁₈ Dynamax (250 mm \times 4.1 mm) from Agilent Technologies. A gradient starting from 20% MeCN / 80% H₂O / 0.1% formic acid and reaching 100% MeCN / 0.1% formic acid at a flow rate of 80 mL/min was used. UV detection (215 and 254 nm) was used to collect the desired product. Purification yields were not optimized.

4.1.6.2. Synthesis of Hit **4** and analogues **15-16**

4.1.6.2.1. 2-(N-[2-[(2S)-4-benzyl-2-(1H-indol-3-ylmethyl)piperazin-1-yl]-2-oxo-ethyl]anilino)acetic acid (**4**)

N-phenyliminodiacetic acid (69 mg, 0.33 mmol, 1 eq.) was put in solution in acetic anhydride (2.5 mL) and trifluoroacetic anhydride (50 μ L) was added. The resulting blackish mixture was stirred at room temperature for 5h. Solvents were then evaporated under reduced pressure. Anhydrous DMF (3 mL) was added on the crude anhydride under argon and the substituted piperidine (100 mg, 0.33 mmol, 1 eq.) was added, followed by DIEA (230 μ L, 1.31 mmol, 4 eq.). The resulting mixture was stirred overnight at room temperature. It was then diluted with water and extracted three times with EtOAc. Organic layers were mixed and solvents were evaporated under reduced pressure. The residue was purified through flash silica gel chromatography (cyclohexane/EtOAc 9:1 to 4:6), affording **4** as an off-white powder (128 mg, 78%). LC tr: 2.96 min, MS (ESI⁺): m/z = 497 [M+H]⁺. Mixture of amide isomers (conformers), approximate ratio: 2.1/1. ¹H NMR (300 MHz, DMSO-d₆) δ : 13.51 (br s, 1H), 11.02 (d, J = 1.6 Hz, 0.6H), 10.76 (d, J = 1.6 Hz, 0.4H), 7.67-7.63 (m, 1H), 7.42-7.26 (m, 6H), 7.23 (d, J = 2.1 Hz, 0.6H), 7.16 (dd, J = 8.4, 7.5 Hz, 0.6H), 7.06-6.89 (m, 3.4H), 6.85 (d, J = 2.1 Hz, 0.4H), 6.67 (t, J = 7.3 Hz, 0.4H), 6.57 (t, J = 7.3 Hz, 0.6H), 6.42 (br d, J = 8.2 Hz, 0.7H), 5.68 (br d, J = 8.2 Hz, 1.3H), 4.64-4.53 (m, 0.7H), 4.33-4.18 (m, 2.3H), 4.11 (s, 0.6H), 3.88-3.82 (m, 1H), 3.72 (s, 0.4H), 3.66-3.47 (m, 3.6H), 3.42-3.26 (m, 1.4H), 3.03 (dd, J = 14.8, 5.0 Hz, 0.6H), 2.96-2.89 (m, 1.6H), 2.82 (dd, J = 13.3, 5.2 Hz, 0.4H), 2.69 (d, J = 11.7 Hz, 0.4H), 2.29 (dd, J = 11.7, 3.4 Hz, 0.6H), 2.22-2.13 (m, 0.4H), 2.02 (td, J = 11.7, 3.5 Hz, 0.6H), 1.88 (dd, J = 11.2, 3.6 Hz, 0.4H). ¹³C NMR (75 MHz, DMSO-d₆) δ : major isomer: 172.4, 169.8, 138.0, 136.3, 128.9, 128.7 (3C), 128.3 (3C), 127.1 (2C), 124.3, 121.1, 118.7, 118.0, 116.6, 111.7, 110.6 (2C), 110.4, 61.9, 54.5 (2C), 53.3, 52.8, 52.6, 37.3, 25.6; minor isomer:

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172.6, 170.0, 146.4, 136.2, 129.1, 128.8 (3C), 128.2 (3C), 127.2 (2C), 123.4, 120.9, 118.3, 118.2, 116.8, 111.3, 111.2 (2C), 110.4, 61.9, 55.9 (2C), 53.1, 52.5, 50.3, 40.7, 25.3.

4.1.6.2.2. *2-[Benzyl-[2-[(2S)-4-benzyl-2-(1H-indol-3-ylmethyl)piperazin-1-yl]-2-oxo-ethyl]amino]acetic acid (15)*

To a stirred solution of commercially available 3-(4-benzyl-piperazin-2-ylmethyl)-1H-indole (250 mg, 0.82 mmol, 1.2 eq.) in anhydrous DMF (8.2 mL) were added DIEA (224 μ L) and N-benzyliminodiacetic anhydride acid (140.2 mg, 0.68 mmol, 1 eq.) in anhydrous DMF (1.4 mL). The mixture was stirred for 5h at room temperature under argon atmosphere and then the solvent was evaporated. The crude product was purified by preparative HPLC to yield **15** as a white solid (260.9 mg, 74%). Purity 100%. LC tr = 3.80 min, MS (ESI+): m/z = 511 [M + H]⁺. ¹H NMR (300 MHz, DMSO-*d*₆) δ : Mixture of conformers. 10.78 (s, 0.5H), 10.68 (s, 0.5H), 7.69 (d, *J* = 7.8Hz, 0.5H), 7.40 (d, *J* = 7.5Hz, 0.5H), 7.37-7.21 (m, 10H), 7.20-7.14 (m, 1H), 7.02 (td, *J* = 7.2 and 0.9 Hz, 1H), 6.99-7.88 (m 1H), 6.88 (d, *J* = 2.4 Hz, 0.5H), 6.78 (d, *J* = 2.1Hz, 0.5H), 4.60 (m, 0.5 H), 4.23 (d, *J* = 13.5 Hz, 0.5H), 4.07 (m, 0.5H), 3.84 (d, *J* = 13.5Hz, 0.5), 3.78 (d, *J* = 13.5Hz, 0.5H), 3.74 (d, *J* = 13.5 Hz, 0.5H), 3.61-3.04 (m, 7H), 3.02-2.59 (m, 4H), 2.06-1.75 (m, 2H).

4.1.6.2.3. *2-[[2-[(2S)-4-benzyl-2-(1H-indol-3-ylmethyl)piperazin-1-yl]-2-oxo-ethyl]-methyl-amino]acetic acid (16)*

To a stirred solution of commercially available 3-(4-benzyl-piperazin-2-ylmethyl)-1H-indole (320 mg, 1.05 mmol, 1.05 eq.) in anhydrous DMF (5 mL) were added DIEA (410 μ L) and N-methyliminodiacetic anhydride acid (1.0 mmol, 1 eq.) in anhydrous DMF (2 mL). The mixture was stirred overnight at room temperature, then the solvent was removed under reduced pressure. The crude product was purified by preparative HPLC to yield **16** as a white solid (42%). Purity 98%. LC tr = 3.03 min, MS (ESI+): m/z = 435 [M + H]⁺. ¹H NMR (300 MHz, DMSO-*d*₆) δ : Mixture of conformers. 10.85 (s, 0.5H), 10.72 (s, 0.5H), 7.70 (d, *J* = 7.5Hz, 0.5H), 7.59 (d, *J* = 7.8Hz, 0.5H), 7.38-7.25 (m, 6H), 7.07-6.92 (m, 2.5H), 6.79 (d, *J* = 1.8 Hz, 0.5H), 4.60 (m, 0.5H), 4.24 (m, 0.5H), 4.16 (m, 0.5H), 3.97-2.68 (m, 12.5H), 2.44 (s, 1.5 H), 2.10 (s, 1.5H), 1.95-1.78 (m, 1H).

4.1.6.3. *Synthesis of Hit 5 and analogues 18-33*

4.1.6.3.1. *Synthesis of sulfonamide derivatives 34a-k*

Procedure A: To a solution of sulfonyl chloride derivative (10 mmol, 1.0 eq.) and triethylamine (12 mmol, 1.2 eq.) in dichloromethane (20 mL) was added the amine derivative (11 mmol, 1.1 eq.) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for 12 h then diluted with dichloromethane (500 mL). The organic phase was washed with aqueous 5% HCl, dried over MgSO₄, filtered and evaporated to give the corresponding sulfonamide.

Procedure B: Sulfonylchloride (2.8 mmol, 1 eq.) was dissolved in 10 mL CH₂Cl₂ and treated with TEA (1.2 mL, 8.5 mmol, 3 eq.) at rt. To this, while stirring the solution, was added amine (04.3 mmol, 1.5 eq.) and the mixture was stirred overnight. The reaction mixture was then poured onto CH₂Cl₂/water mixture and washed once. The aqueous phase was back extracted once with fresh CH₂Cl₂. Organic phases were combined, washed once with brine and dried over MgSO₄ then filtered and concentrated *in vacuo*.

[Tapez ici]

4.1.6.3.2. *N*-(2-phenylethyl)benzenesulfonamide **34a**

Obtained following the general procedure A. Yellow solid (2.34 g, 90%). Mp: 61.5-68.5 °C. LC tr = 5.8 min. MS (ESI+): m/z = 262 [M+H]⁺. ¹H NMR (300 MHz, CDCl₃) δ: 7.81 (m, 2H), 7.54 (m, 3H), 7.25 (m, 3H), 7.08 (m, 2H), 4.68 (br s, 1H), 3.24 (m, 2H), 2.77 (t, *J* = 6.9 Hz, 2H). ¹³C NMR (75 MHz, CDCl₃) δ: 139.9, 137.6, 132.7, 129.1, 128.8, 127.0, 126.8, 44.3, 35.8.

4.1.6.3.3. 4-fluoro-*N*-(2-phenylethyl)benzenesulfonamide **34b**

Obtained following the general procedure A. Yellow solid (2.45 g, 88%). Mp: 83-84.6 °C. LC tr = 6.0 min. MS (ESI+): m/z = 280 [M+H]⁺. ¹H NMR (300 MHz, CDCl₃) δ: 7.81 (m, 2H), 7.25 (m, 3H), 7.16 (m, 2H), 7.08 (m, 2H), 4.49 (br s, 1H), 3.23 (m, 2H), 2.78 (t, *J* = 6.9 Hz, 2H). ¹³C NMR (75 MHz, CDCl₃) δ: 129.6 (d, *J*_{C-F} = 36 Hz), 128.6, 128.5, 126.7, 116.1 (d, *J*_{C-F} = 90 Hz), 44.0, 35.5.

4.1.6.3.4. *N*-(2-phenylethyl)methanesulfonamide **34c**

Obtained following the general procedure A. Yellow solid (1.78 g, 98%). Mp: 45.7-48.5 °C. LC tr = 4.1 min. MS (ESI+): m/z = 200 [M+H]⁺. ¹H NMR (300 MHz, CDCl₃) δ: 7.30 (m, 2H), 7.25 (m, 1H), 7.22 (m, 2H), 4.37 (br s, 1H), 3.41 (m, 2H), 2.88 (t, *J* = 6.9 Hz, 2H), 2.82 (s, 3H). ¹³C NMR (75 MHz, CDCl₃) δ: 129.0, 127.1, 44.6, 40.5, 36.6.

4.1.6.3.5. 4-fluoro-*N*-(2-pyrrolidin-1-ylethyl)benzenesulfonamide **34d**

Obtained following the general procedure A. Yellow solid (2.03 g, 75%). Mp: 55.3-60.2 °C. LC tr = 2.5 min. MS (ESI+): m/z = 273 [M+H]⁺. ¹H NMR (300 MHz, CDCl₃) δ: 7.90 (m, 2H), 7.18 (m, 2H), 5.23 (br s, 1H), 3.06 (t, *J* = 6 Hz, 2H), 2.68 (t, *J* = 6 Hz, 2H), 2.55 (m, 4H), 1.78 (m, 4H). ¹³C NMR (75 MHz, CDCl₃) δ: 129.9 (d, *J*_{C-F} = 30 Hz, 2C), 116.4 (d, *J*_{C-F} = 90 Hz, 2C), 54.2, 53.8 (2C), 41.0, 23.5 (2C).

4.1.6.3.6. *N*-(2-pyrrolidin-1-ylethyl)methanesulfonamide **34e**

Obtained following the general procedure A. Yellow solid (0.71 g, 37%). Mp: 55.0-58.2 °C. LC tr = 5.5 min. MS (ESI+): m/z = 193 [M+H]⁺. ¹H NMR (300MHz, CDCl₃) δ: 3.24 (t, *J* = 5.7 Hz, 2H), 2.92 (s, 3H), 2.74 (t, *J* = 5.7 Hz, 2H), 2.62 (m, 4H), 1.81 (m, 4H). ¹³C NMR (75MHz, CDCl₃) δ: 54.8, 53.9 (2C), 41.4, 40.1, 23.6 (2C).

4.1.6.3.7. *N*-(2-(pyrrolidin-1-yl)ethyl)benzenesulfonamide **34f**

Obtained from procedure B. The product was obtained in quantitative yield and used in the next step without further purification. Purity: 95%. LC tr = 1.52 min. MS (ESI+): m/z = 253 [M-H]⁻. ¹H NMR (300 MHz, CDCl₃) δ: 7.86-7.88 (m, 2H), 7.49-7.57 (m, 3H), 4.67 (s, br, 1H), 3.02 (t, *J* = 5.8 Hz, 2H), 2.56 (t, *J* = 5.8 Hz, 2H), 2.37-2.42 (m, 4H), 1.70-1.74 (m, 4H). ¹³C NMR (75 MHz, CDCl₃) δ: 139.8, 132.7, 129.2 (2C), 127.2 (2C), 53.9, 53.6 (2C), 41.2, 23.6 (2C).

4.1.6.3.8. 4-fluoro-*N*-(3-(pyrrolidin-1-yl)propyl)benzenesulfonamide **34g**

Obtained from procedure B. The residue was purified through flash silica gel column (CH₂Cl₂ to CH₂Cl₂/MeOH 95:5) to give the 4-fluoro-*N*-(3-(pyrrolidin-1-yl)propyl)benzenesulfonamide as an orange oil (465 mg, 63%). Purity: 90%, LC t_R = 1.52 min, MS (ESI+): m/z = 287 [M + H]⁺. ¹H NMR (300MHz, CD₃OD) δ: 7.93-7.86 (m, 2H), 7.35-7.27 (m, 2H), 2.91 (t, *J* = 6.9 Hz, 2H), 2.53-2.45 (m, 6H), 1.84-1.76 (m, 4H), 1.74-1.62 (m, 2H). ¹³C NMR (75 MHz, CD₃OD) δ: 166.3 (*J* = 252 Hz), 138.2 (*J* = 3 Hz), 130.9 (*J* = 9 Hz, 2C), 117.2 (*J* = 23 Hz, 2C), 54.9 (2C), 54.7, 42.5, 29.5, 24.1 (2C).

4.1.6.3.9. *N*-(2-(diethylamino)ethyl)-4-fluorobenzenesulfonamide **34h**

[Tapez ici]

Obtained from procedure B. The residue was purified through flash silica gel column (CH₂Cl₂ to CH₂Cl₂/MeOH 95:5) to give the N-[2-(diethylamino)ethyl]-4-fluoro-benzenesulfonamide as a yellow oil (498 mg, 71%). Purity: 100%, LC t_R = 1.65 min, MS (ESI-): m/z = 273 [M - H]⁻. ¹H NMR (300MHz, CD₃OD) δ: 7.95-7.88 (m, 2H), 7.35-7.27 (m, 2H), 2.98-2.93 (m, 2H), 2.56-2.46 (m, 6H), 0.98 (t, J = 7.2 Hz, 6H). ¹³C NMR (75MHz, CD₃OD) δ: 166.3 (J = 252 Hz), 138.1 (J = 3 Hz), 130.9 (J = 10 Hz, 2C), 117.2 (J = 23 Hz, 2C), 53.0, 48.0 (2C), 41.4, 11.5 (2C).

4.1.6.3.10. N-(2-(dimethylamino)ethyl)-4-fluorobenzenesulfonamide 34i

Obtained from procedure B. The residue was purified through flash silica gel column (CH₂Cl₂ to CH₂Cl₂/MeOH 95:5) to give the N-[2-(dimethylamino)ethyl]-4-fluoro-benzenesulfonamide as a yellow oil (505 mg, 80%). Purity: 100%, LC t_R = 1.50 min, MS (ESI-): m/z = 245 [M - H]⁻. ¹H NMR (300MHz, CD₃OD) δ: 7.95-7.88 (m, 2H), 7.35-7.27 (m, 2H), 2.97 (t, J = 6.8 Hz, 2H), 2.40 (t, J = 6.7 Hz, 2H), 2.18 (s, 6H). ¹³C NMR (75MHz, CD₃OD) δ: 166.4 (J = 252 Hz), 138.0 (J = 3 Hz), 131.0 (J = 9 Hz, 2C), 117.2 (J = 23 Hz, 2C), 59.4, 45.4 (2C), 41.6.

4.1.6.3.11. 4-fluoro-N-(2-(piperidin-1-yl)ethyl)benzenesulfonamide 34j

Obtained from procedure B. The residue was purified by flash chromatography on silica gel (CH₂Cl₂/MeOH, 100:0 to 95:5 (v/v)) to give the 4-fluoro-N-[2-(1-piperidyl)ethyl]benzenesulfonamide as a yellow oil (185 mg 78%). Purity: 99%, LC t_R = 1.65 min, MS (ESI+): m/z = 287 [M + H]⁺ and MS (ESI): m/z = 285 [M - H]⁻. ¹H NMR (300MHz, CD₃OD) δ: 7.95-7.88 (m, 2H), 7.35-7.27 (m, 2H), 3.02-2.97 (m, 2H), 2.43-2.36 (m, 6H), 1.59-1.51 (m, 4H), 1.46-1.43 (m, 2H). ¹³C NMR (75MHz, CD₃OD) δ: 166.6 (J = 256 Hz), 138.1 (J = 3 Hz), 131 (J = 9 Hz), 117.2 (J = 23 Hz), 59.0, 55.4, 41.0, 26.5, 25.0.

4.1.6.3.12. 4-fluoro-N-(2-morpholinoethyl)benzenesulfonamide 34k

Obtained from procedure B. The residue was purified through flash silica gel column (CH₂Cl₂ to CH₂Cl₂/MeOH 95:5) to give the 4-fluoro-N-(2-morpholinoethyl)benzenesulfonamide as a red solid (582 mg, 79%). Purity: 100%, LC t_R = 1.70 min, MS (ESI+): m/z = 289 [M + H]⁺. ¹H NMR (300MHz, CD₃OD) δ: 7.95-7.88 (m, 2H), 7.35-7.27 (m, 2H), 3.64-3.61 (m, 4H), 3.10 (t, J = 6.7 Hz, 2H), 2.42 (t, J = 6.7 Hz, 2H), 2.39-2.36 (m, 4H). ¹³C NMR (75MHz, CD₃OD) δ: 166.4 (J = 253 Hz), 138.2 (J = 3 Hz), 131 (J = 10 Hz, 2C), 117.2 (J = 23 Hz, 2C), 67.6 (2C), 58.7, 54.6 (2C), 40.8.

4.1.6.3.13. General procedure C for the synthesis of 5b, 18b-19b.

To a solution of 4-iodobenzoyl chloride (5.6 mmol, 1.0 eq.) in dichloromethane (30 mL) was added triethylamine (22.4 mmol, 4.0 eq.) and t-butyl ester of amino acid (5.6 mmol, 1.0 eq.) respectively. The mixture was stirred at room temperature for 4 h then diluted with dichloromethane (150 mL). The organic phase washed with aqueous saturated NaHCO₃ and aqueous 0.1 N KHSO₄, dried over MgSO₄, filtered and evaporated. The crude product was purified by flash chromatography on silica gel (with a gradient in cyclohexane/ethyl acetate or CH₂Cl₂/MeOH) to afford the pure product.

4.1.6.3.14. tert-butyl (2S)-2-[(4-iodobenzoyl)amino]-3-phenyl-propanoate (5b).

Obtained following the general procedure C. Light brown solid (2.22 g, 88%). Mp: 116.1-119.8 °C. LC t_R = 7.5 min. MS (ESI+): m/z = 396 [M+H-t-Bu]⁺. ¹H NMR (300 MHz, CDCl₃) δ: 7.75 (m, 2H), 7.45 (m, 2H), 7.26 (m, 3H), 7.17 (m, 2H), 5.29 (br s, 1H), 4.93 (m, 1H), 3.22 (m, 2H), 1.45 (s, 9H). ¹³C NMR (75 MHz, CDCl₃) δ: 170.0, 165.9, 137.7, 136.0, 133.4, 129.5, 128.5 (2C), 128.4 (2C), 127.0, 127.0, 98.7, 82.7, 53.9, 53.9, 37.9, 28.0 (3C).

[Tapez ici]

4.1.6.3.15. *tert-butyl (2S)-2-[(4-iodobenzoyl)amino]-4-methyl-pentanoate (18b)*.

Obtained following the general procedure C. Light brown solid (2.20 g, 94%). LC tr = 7.7 min. MS (ESI⁻): m/z = 416 [M-H]⁻. ¹H NMR (300 MHz, CDCl₃) δ: 7.77 (d, *J* = 8.5 Hz, 2H) 7.51 (d, *J* = 8.5 Hz, 2H), 6.59 (d, *J* = 8.3 Hz, 1H), 4.71 (td, *J* = 5.3, 8.3 Hz, 1H), 1.58-1.76 (m, 3H), 1.48 (s, 9H), 0.96-0.99 (m, 6H). ¹³C NMR (75 MHz, CDCl₃) δ: 172.6, 166.3, 137.9 (2C), 133.7, 128.8 (2C), 98.7, 82.4, 51.9, 42.3, 28.2 (3C), 25.2, 23.0, 22.4.

4.1.6.3.16. *(S)-tert-butyl 2-(4-iodobenzamido)-4-phenylbutanoate (19b)*

Obtained following the general procedure C. Yellow solid (2.42 g, 95%). Purity: 98%, LC t_R = 3.52 min, MS (ESI⁺): m/z = 466 [M + H]⁺. ¹H NMR (300MHz, CDCl₃) δ: 7.76 (d, *J* = 8.5 Hz, 2H), 7.41 (d, *J* = 8.5 Hz, 2H), 7.31-7.26 (m, 2H), 7.21-7.18 (m, 3H), 6.66 (d, *J* = 7.4 Hz, 1H), 4.80-4.73 (m, 1H), 2.79-2.63 (m, 2H), 2.36-2.24 (m, 1H), 2.17-2.05 (m, 1H), 1.51(s, 9H). ¹³C NMR (75MHz, CDCl₃) δ: 171.7, 166.1, 141.2, 137.8 (2C), 133.6, 128.7 (4C), 128.5 (2C), 126.3, 98.8, 82.8, 53.3, 34.3, 31.8, 28.2 (3C).

4.1.6.3.17. *General procedure D for the synthesis of 5c, 18c-19c*

To a suspension of sodium hydride (4.6 mmol, 1.3 eq.) in anhydrous THF (11 mL) was added amide compound (5b, 18-26b, 28b-33b) (3.5 mmol, 1.0 eq.) at 0°C. The reaction mixture was stirred for 1 h and iodomethane (7.0 mmol, 2.0 eq.) was added. The mixture was stirred at room temperature overnight. Water (200 mL) was then added and the resulting mixture was extracted with EtOAc. The organic phases were combined, dried over MgSO₄ and evaporated to give the corresponding methylated product. The crude product was purified by flash chromatography on silica gel (with a gradient in cyclohexane/ethyl acetate or CH₂Cl₂/MeOH) to afford the pure product.

4.1.6.3.18. *tert-butyl (2S)-2-[(4-iodobenzoyl)-methyl-amino]-3-phenyl-propanoate (5c)*.

Obtained following the general procedure D. Yellow oil (1.33 g, 82%). LC tr = 7.9 min. MS (ESI⁺): m/z = 410 [M+H]⁺. ¹H NMR (300 MHz, CDCl₃) δ: Mixture of 2 cis/trans amide isomers (ratio 1/0.9) 7.68 (d, *J* = 8.1 Hz, 2H), 7.56 (d, *J* = 8.1 Hz, 2H), 7.29-7.28 (m, 8H), 7.01 (m, 2H), 6.84 (d, *J* = 7.8 Hz, 2H), 6.47 (d, *J* = 7.8 Hz, 2H), 4.34 (m, 1H), 3.46 (m, 1H), 3.18-2.93 (m, 7H), 2.74 (s, 3H), 1.52 (s, 18H). ¹³C NMR (75 MHz, CDCl₃) δ: 172.1 (maj), 171.2 (min), 169.8 (min), 169.2 (maj), 137.6 (2C min), 137.4 (2C maj), 136.8, 135.7 (min), 135.3 (maj), 129.4 (2C), 128.9 (4C), 128.9 (4CH), 128.7 (2C min), 128.6 (2C min), 128.4 (2C maj), 127.2 (maj), 126.9 (min), 95.8 (min), 95.5 (maj), 82.9 (maj), 82.1 (min), 64.2 (maj), 58.8 (min), 35.3 (min), 34.7, 29.3 (maj), 28.2 (3C).

4.1.6.3.19. *tert-butyl (2S)-2-[(4-iodobenzoyl)-methyl-amino]-4-methyl-pentanoate (18c)*.

Obtained following the general procedure D. White solid (1.16 g, 77%). LC tr = 8.1 min. MS (ESI⁺): m/z = 376 [M+H-*t*Bu]⁺. ¹H NMR (300 MHz, CDCl₃) δ: 7.73-7.75 (m, 4H), 7.14-7.17 (m, 4H), 5.24 (dd, *J* = 6.2, 9.3 Hz, 1H), 4.17 (dd, *J* = 6.0, 8.9 Hz, 1H), 2.96 (s, 3H), 2.84 (s, 3H), 1.61-1.77 (m, 6H), 1.48 (s, 18H), 0.99-1.00 (m, 6H), 0.86 (d, *J* = 6.3 Hz, 3H), 0.66 (d, *J* = 6.3 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ: 171.9, 171.5, 170.8, 170.3, 137.7 (4C), 136.1, 135.8, 128.7 (2C), 128.7 (2C), 96.0, 95.8, 82.5, 81.8, 60.7, 55.4, 38.1, 37.2, 33.9, 29.2, 28.2 (3C), 28.2 (3C), 25.4, 24.7, 23.5, 23.2, 21.7, 21.7.

4.1.6.3.20. *(S)-tert-butyl 2-(4-iodobenzamido)-4-phenylbutanoate (19c)*

Obtained following the general procedure D. White solid (1.0 g, 58%). Purity: 95%, LC t_R = 3.67 min, MS (ESI⁺): m/z = 480 [M + H]⁺. Mixture of 2 amide conformers with a ratio of 1/0.8. ¹H NMR (300MHz, CDCl₃) δ: 7.76 (d, *J* = 7.8 Hz, 2H), 7.65 (d, *J* = 7.8 Hz, 2H), 7.33-7.28 (m, 2H), 7.23-7.14 (m, 8H),

[Tapez ici]

7.07-7.00 (m, 4H), 5.14 (dd, $J = 4.7, 10.2$ Hz, 1H), 4.17 (dd, $J = 6.0, 8.9$ Hz, 1H), 3.01 (s, 3H), 2.86 (s, 3H), 2.78-2.62 (m, 2H), 2.55-2.47 (m, 2H), 2.43-2.22 (m, 2H), 2.15-1.90 (m, 2H), 1.48 (s, 18H). ^{13}C NMR (75MHz, CDCl_3) δ : 172.0, 171.6, 170.1, 169.7, 141.1, 140.2, 137.7 (4C), 135.9, 135.6, 128.8 (2C), 128.7 (4C), 128.5 (2C), 128.5 (2C), 128.3 (2C), 126.5, 126.4, 96.0, 95.8, 82.7, 82.0, 61.6, 57.4, 34.2, 33.1, 32.2, 30.9, 30.3, 29.0, 28.2 (6C).

4.1.6.3.21. General procedure E and E' for the arylation of sulfonamides

Procedure E: To a mixture of CuI (0.015 mmol, 0.1 eq.) and K_3PO_4 (0.3 mmol, 2eq.) under argon was added a solution of sulfonamide derivative (0.165 mmol, 1.1 eq.) and iodo derivative (0.15 mmol, 1 eq.) in anhydrous dioxane (0.3 mL) and trans-1,2-diaminocyclohexane (0.03 mmol, 0.2 eq.) respectively. The reaction mixture was stirred at 110 °C for 36 h. Water (30 mL), aqueous 20% ammonia (1 mL) and aqueous 10% NaOH were then added. The resulting mixture was extracted with EtOAc. The organic layers were combined, washed with aqueous saturated NaHCO_3 and 0.1 N KHSO_4 , filtered and evaporated. The product was used directly in the next step.

Procedure E': To a mixture of CuI (0.03 mmol, 0.1 equiv), K_2CO_3 (0.63 mmol, 2.5 equiv), sulfonamide derivative (0.30 mmol, 1.2 equiv), iodo derivative (0.25 mmol, 1 equiv) and N,N'-dimethylethane-1,2-diamine (20 μL , 0.18 mmol, 0.7 equiv) was added anhydrous ACN (1.5 mL) under argon atmosphere. The reaction mixture was heated at 80 °C for 48h. After cooling down to rt, water was then added and the resulting mixture was extracted with EtOAc. The organic layers were combined, washed with aqueous saturated NaHCO_3 and 0.1 N KHSO_4 , filtered and evaporated. The product was used directly in the next step.

4.1.6.3.22. General déprotection procedures F and F'

Procedure F: To a solution of *t*-butyl derivative (0.1 mmol, 1.0 eq.) in CH_2Cl_2 (0.95 mL) was added triisopropylsilane (0.1 mL) and trifluoroacetic acid (0.95 mL) respectively. The reaction mixture was stirred at room temperature for 1 h then evaporated. The crude was taken in a mixture of Et_2O /pentane (200 μL /300 μL) then filtered. The filtrate was evaporated to give the desired product.

Procedure F': To a solution of the tert-butyl derivative (0.1 mmol, 1.0 eq.) in CH_2Cl_2 (2 mL) was added HCl (4N solution in dioxane, 2 mL). The mixture was then stirred at room temperature for 24h. The mixture was diluted with EtOAc and 1.1 mL of NEt_3 was added. The solvents were then evaporated and the crude was purified by flash chromatography on normal or reverse phase silica gel (with a gradient of cyclohexane/ethyl acetate or CH_2Cl_2 /MeOH, or water/MeOH) to afford the pure product.

4.1.6.3.23. (2S)-2-[[4-[(4-fluorophenyl)sulfonyl-(2-pyrrolidin-1-ylethyl)amino]benzoyl]-methyl-amino]-3-phenyl-propanoic acid (5).

5d was obtained following the general procedure E. Colorless oil (68 mg, 74%). LC $t_R = 5.8$ min. MS (ESI+): $m/z = 610$ $[\text{M}+\text{H}]^+$. ^1H NMR (300 MHz, CDCl_3) δ : Mixture of 2 cis/trans amide isomers (ratio 1/0.9) 7.60-7.56 (m, 4H), 7.33-7.26 (m, 8H), 7.16-7.11 (m, 4H), 7.06 (m, 4H), 6.97-6.91 (m, 4H), 6.74-6.71 (m, 2H), 5.30 (m, 1H), 4.33 (m, 1H), 3.89 (m, 4H), 3.46 (m, 1H), 3.15 (m, 6H), 2.94 (m, 12H), 2.74 (s, 3H), 1.95 (m, 8H), 1.52 (s, 18H). ^{13}C NMR (75 MHz, CDCl_3) δ : 171.7, 170.8, 169.6, 169.0, 165.4 (d, $J_{\text{C-F}} = 255$ Hz, 2C), 139.9, 139.7, 137.2, 136.7, 136.2, 135.7, 133.2 (2C), 130.4 (d, $J_{\text{C-F}} = 10$ Hz, 4C), 129.2 (2C), 128.8 (2C), 128.4 (2C), 128.2 (2C), 127.9 (2C), 127.7 (2C), 127.1, 126.8, 116.3 (d, $J_{\text{C-F}} =$

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23 Hz, 4C), 82.9, 82.1, 64.2, 58.8, 54.1 (4C), 48.3, 48.2, 35.3, 34.6, 34.5, 29.3, 28.0 (6C), 23.4 (4C).

Then **5**

was obtained following the general procedure F (25 mg, 45%). LC tr = 4.3 min. MS (ESI+): m/z = 554 [M+H]⁺. ¹H NMR (300 MHz, CD₃OD) δ: Mixture of 2 cis/trans amide isomers (ratio 1/0.9) 7.67-6.71 (m, 26H), 5.30 (dd, J = 5.1, 11.6 Hz, 1H), 4.46 (dd, J = 3.8, 11.1 Hz, 1H), 4.00-3.76 (m, 8H), 3.48 (dd, J = 4.8, 14.5 Hz, 1H), 3.25-3.08 (m, 14H), 2.77 (s, 3H), 2.14-2.09 (m, 8H). ¹³C NMR (75 MHz, CD₃OD) δ: 173.8, 173.3, 173.1, 172.6, 167.2 (d, J_{C-F} = 254.5 Hz, 2C), 140.9, 140.6, 138.9, 138.6, 137.4, 137.1, 134.0 (d, J_{C-F} = 3.1 Hz, 2C), 132.0 (d, J_{C-F} = 9.3 Hz, 4C), 130.4 (2C), 130.1 (2C), 129.8 (2C), 129.8 (2C), 129.6 (2C), 129.5 (2C), 128.9 (2C), 128.7 (2C), 128.1, 127.9, 117.6 (d, J_{C-F} = 22.9 Hz, 4C), 65.3, 60.4, 55.9 (4C), 54.2, 54.1, 48.1 (2C), 36.5, 35.6, 35.3, 30.2, 24.0 (4C).

4.1.6.3.24. *2S)-2-[[4-[(4-fluorophenyl)sulfonyl-(2-pyrrolidin-1-ylethyl)amino]benzoyl]-methyl-amino]-4-methyl-pentanoic acid (18).*

18d was obtained following the general procedure E (85 mg, 98%). t_{R,LCMS} = 5.6 min. MS (ESI+): m/z = 576 [M+H]⁺, and was used without further purification. **18** was obtained from **18d** following the general procedure F (21 mg, 40%). LC tr = 4.3 min. MS (ESI+): m/z = 520 [M+H]⁺. ¹H NMR (300 MHz, CD₃OD) δ: Mixture of 2 cis/trans amide isomers (ratio 1/0.8) 7.7.70-7.56 (m, 4H), 7.50-7.38 (m, 4H), 7.37-7.22 (m, 8H), 5.25 (dd, J = 4.9, 10.8 Hz, 1H), 4.27 (dd, J = 5.1, 9.9 Hz, 1H), 3.38-3.32 (m, 4H), 3.68-3.85 (m, 4H), 3.24-3.08 (m, 4H), 3.00 (s, 3H), 2.93 (s, 3H), 2.24-2.98 (m, 8H), 1.48 (s, 1H), 1.97-1.72 (m, 4H), 1.71-1.57 (m, 1H), 1.56-1.39 (m, 1H), 1.03 (d, J = 7.4 Hz, 3H), 1.01 (d, J = 6.7 Hz, 3H), 0.91 (d, J = 6.7 Hz, 3H), 0.69 (d, J = 6.5 Hz, 3H). ¹³C NMR (75 MHz, CD₃OD) δ: 174.3, 173.8, 173.6, 173.5, 167.2 (d, ¹J_{C-F} = 254 Hz, 2C), 141.1 (2C), 137.6, 137.5, 134.0, 133.8, 132.0 (d, J_{C-F} = 9.7 Hz, 2C), 131.9 (d, J_{C-F} = 9.6 Hz, 2C), 130.0 (2C), 129.9 (2C), 129.2 (2C), 128.9 (2C), 117.6 (d, ²J_{C-F} = 22.9 Hz, 4C), 61.5, 56.4, 55.9 (4C), 54.3, 54.2, 39.0, 38.1, 34.7, 29.8, 26.4, 25.8, 24.0 (4C), 23.6, 23.3, 21.9, 21.6.

4.1.6.3.25. *(S)-2-(4-(4-fluoro-N-(2-(pyrrolidin-1-yl)ethyl)phenylsulfonamido)-N-methylbenzamido)-4-phenylbutanoic acid (19)*

19d was obtained following the general procedure E': Purity: 100% LC t_R = 3.08 min. MS (ESI+): m/z = 624 [M+H]⁺. ¹H NMR (300 MHz, CDCl₃) δ: Mixture of 2 amide conformers with a ratio of 1/0.8. 7.57-7.64 (m, 4H), 7.37 (d, J = 8.0 Hz, 2H), 7.20-7.29 (m, 10H), 7.03-7.14 (m, 10H), 5.13 (dd, J = 4.8, 10.4 Hz, 1H), 4.17 (dd, J = 4.9, 9.7 Hz, 1H), 3.67-3.72 (m, 4H), 3.04 (s, 3H), 2.88 (s, 3H), 2.67-2.76 (m, 2H), 2.57-2.62 (m, 5H), 2.25-2.50 (m, 11H), 2.00-2.12 (m, 2H), 1.72-1.75 (m, 8H), 1.47 (m, 18H). ¹³C NMR (75 MHz, CDCl₃) δ: 172.0, 171.5, 170.0, 169.7, 165.3 (d, J_{C-F} = 255 Hz, 2C), 141.0, 140.5, 140.4, 140.1, 135.9, 135.7, 134.2 (d, J_{C-F} = 3 Hz, 2C), 130.4 (d, J_{C-F} = 9 Hz, 4C), 128.8 (4C), 128.7 (2C), 128.7 (2C), 128.5 (2C), 128.2 (2C), 128.0 (2C), 127.8 (2C), 126.5, 126.3, 116.2 (d, J_{C-F} = 23 Hz, 4C), 82.7, 82.0, 61.8, 57.4, 54.4 (2C), 54.2 (4C), 49.8, 49.8, 34.3, 33.0, 32.2, 30.7, 30.2, 29.1, 28.2 (3C), 28.1 (3C), 23.6 (4C). **19** was obtained from **19d** following the general procedure F': White solid (27 mg, 62%). Purity: 100%, LC t_R = 2.28 min, MS (ESI+): m/z = 568 [M + H]⁺. HRMS m/z calculated for C₃₀H₃₅FN₃O₅S [M+H]⁺ 568.2281 found 568.2299. Mixture of 2 amide conformers with a ratio of 1/0.4. ¹H NMR (300 MHz, CD₃OD) δ: 7.67-7.56 (m, 4H), 7.46 (d, J = 8.0 Hz, 2H), 7.38 (d, J = 7.7 Hz, 2H), 7.32-7.11 (m, 18H), 5.01 (dd, J = 3.9, 10.9 Hz, 1H), 4.06-3.93 (m, 5H), 3.26-3.14 (m, 12H), 3.10

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(s, 3H), 2.89 (s, 3H), 2.73-2.58 (m, 3H), 2.45-2.34 (m, 3H), 2.16-2.03 (m, 10H). ¹³C NMR (75 MHz, CD₃OD) δ: 177.3, 173.8 (2C), 173.3, 167.0 (d, *J*_{C-F} = 253 Hz, 2C), 142.9, 142.2, 141.0, 140.7, 138.3, 137.9, 134.4 (d, *J*_{C-F} = 3 Hz), 134.3 (d, *J*_{C-F} = 3 Hz), 132.0 (d, *J*_{C-F} = 10 Hz, 4C), 129.8 (2C), 129.6 (4C), 129.5 (2C), 129.4 (2C), 129.4 (2C), 129.2 (4C), 127.1, 127.0, 117.5 (d, *J*_{C-F} = 23 Hz, 4C), 65.2, 60.3, 55.5 (2C), 55.4 (2C), 54.3 (2C), 48.6 (2C), 34.8, 34.5, 33.9, 32.5, 32.2, 30.2, 24.1 (4C).

4.1.6.3.26. (2*S*)-2-[methyl-[4-[methylsulfonyl(2-pyrrolidin-1-ylethyl)amino]benzoyl]amino]-3-phenylpropanoic acid (**20**)

20d was obtained following the general procedure E (64 mg, 80%). *t*_{R,LCMS} = 5.0 min. MS (ESI⁺): *m/z* = 530 [M+H]⁺. Then, **20** was obtained from **20d** following the general procedure F: (10 mg, 21%). LC *t*_R = 3.4 min. MS (ESI⁺): *m/z* = 474 [M+H]⁺. ¹H NMR (300 MHz, CD₃OD) δ: Mixture of 2 cis/trans amide isomers (ratio 1/0.9) 7.54 (d, *J* = 8.5 Hz, 2H), 7.43 (d, *J* = 8.7 Hz, 2H), 7.35-7.28 (m, 8H), 7.17 (d, *J* = 8.5 Hz, 2H), 7.05-7.02 (m, 2H), 6.83 (d, *J* = 8.2 Hz, 2H), 5.31 (dd, *J* = 4.8, 11.7 Hz, 1H), 4.55 (dd, *J* = 4.0, 11.6 Hz, 1H), 4.12 (t, *J* = 5.7 Hz, 4H), 3.73 (m, 4H), 3.49 (dd, *J* = 4.8, 14.2 Hz, 1H), 3.27-3.08 (m, 14H), 3.01-3.00 (m, 6H), 2.79 (s, 3H), 2.09 (m, 8H). ¹³C NMR (75 MHz, CD₃OD) δ: 173.9, 173.4, 173.1, 172.7, 141.4, 141.1, 138.9, 138.6, 137.4, 137.0, 130.4 (2C), 130.1 (2C), 129.8 (2C), 129.8 (2C), 129.6 (2C), 129.5 (2C), 129.1 (2C), 129.1 (2C), 128.1, 127.9, 65.2, 60.4, 55.8 (4C), 54.4 (2C), 48.2 (2C), 36.6 (2C), 36.5, 35.6, 35.3, 30.3, 23.9 (4C).

4.1.6.3.27. (S)-2-(*N*-methyl-4-(*N*-(2-(pyrrolidin-1-yl)ethyl)phenylsulfonamido)benzamido)-3-phenylpropanoic acid (**21**).

21d was obtained following the general procedure E': Purity: 100% LC *t*_R = 2.83 min. MS (ESI⁺): *m/z* = 592 [M+H]⁺. ¹H NMR (300 MHz, CDCl₃) δ: Mixture of 2 amide conformers with a ratio of 1/0.9. 7.54-7.60 (m, 6H), 7.44-7.48 (m, 4H), 7.21-7.31 (m, 8H), 7.03 (m, 4H), 6.91-6.93 (m, 4H), 6.69-6.72 (m, 2H), 5.30 (dd, *J* = 5.0, 11.1 Hz, 1H), 4.35 (dd, *J* = 3.9, 11.1 Hz, 1H), 3.57-3.74 (m, 4H), 3.45 (dd, *J* = 5.0, 14.9 Hz, 1H), 3.09-3.17 (m, 2H), 3.05 (s, 3H), 2.89-2.98 (m, 1H), 2.73 (s, 3H), 2.47-2.56 (m, 12H), 1.72-1.73 (m, 8H), 1.50 (s, 18H). ¹³C NMR (75 MHz, CDCl₃) δ: 172.0, 171.2, 169.8, 169.2, 140.5, 140.2, 138.2 (2C), 137.4, 136.8, 135.7, 135.2, 132.9 (4C), 129.2 (2C), 129.0 (4C), 128.9 (2C), 128.8 (2C), 128.7 (2C), 128.5 (2C), 127.7 (4C), 127.5 (2C), 127.1, 126.9, 82.9, 82.1, 64.2, 58.8, 54.4 (2C), 54.3 (4C), 49.8 (2C), 35.4, 34.7, 34.7, 29.4, 28.1 (6C), 23.6 (4C). Then **21** was obtained from **21d** following the general procedure F'. White solid (28 mg, 57%). Purity: 100%, LC *t*_R = 2.05 min, MS (ESI⁺): *m/z* = 536 [M + H]⁺. HRMS *m/z* calculated for C₂₉H₃₄N₃O₅S [M+H]⁺ 536.2219, found 536.2209. Mixture of 2 amide conformers with a ratio of 1/0.3. ¹H NMR (300 MHz, CD₃OD) δ: 7.71-7.66 (m, 2H), 7.61-7.52 (m, 8H), 7.31-7.26 (m, 2H), 7.19-7.18 (m, 6H), 7.09 (d, *J* = 7.9 Hz, 2H), 7.02-6.9 (m, 6H), 6.78 (d, *J* = 7.7 Hz, 2H), 5.40 (dd, *J* = 4.4, 11.4 Hz, 1H), 4.39-4.34 (m, 1H), 4.06-3.82 (m, 4H), 3.53 (dd, *J* = 4.5, 14.7 Hz, 1H), 3.39-3.34 (m, 1H), 3.24-3.09 (m, 16H), 2.97 (dd, *J* = 11.8, 13.6 Hz, 1H), 2.76 (s, 3H), 2.03 (m, 8H). ¹³C NMR (75 MHz, CD₃OD) δ: 177.1, 176.3, 173.8, 173.0, 140.8, 140.5, 140.4, 140.3, 138.2 (2C), 137.5 (2C), 134.7 (2C), 130.4 (4C), 130.3 (2C), 130.0 (2C), 129.6 (4C), 129.4 (2C), 129.3 (2C), 129.0 (2C), 128.8 (4C), 128.7 (2C), 127.5, 127.4, 68.1, 61.4, 55.4 (4C), 54.4 (2C), 48.7 (2C), 36.9, 36.6, 35.3, 30.7, 24.1 (4C).

4.1.6.3.28. (S)-2-(4-(4-fluoro-*N*-(3-(pyrrolidin-1-yl)propyl)phenylsulfonamido)-*N*-methylbenzamido)-3-phenylpropanoic acid (**22**)

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22d was obtained following the general procedure E'. Purity: 85%. LC t_R = 3.05 min, MS (ESI+): m/z = 624 [M + H]⁺. Complex mixture of 2 amide conformers with a ratio of 1 (maj) / 0.9 (min). ¹H NMR (300MHz, CDCl₃) δ : 7.62-7.58 (m, 4H), 7.36-7.29 (m, 4H), 7.18-7.08 (m, 9H), 7.03-7.00 (m, 4H), 6.91 (d, J = 8.1 Hz, 2H), 6.75 (d, J = 7.6 Hz, 2H), 5.30 (dd, J = 5.1 and 11.3 Hz, 1H), 4.35 (dd, J = 3.8 and 10.7 Hz, 1H), 3.74-3.70 (m, 4H), 3.53-2.91 (m, 21H), 2.76 (s, 3H), 2.12-2.02 (m, 10H), 1.52 (s, 18H). ¹³C NMR (75MHz, CDCl₃) δ : 171.7, 170.9, 169.3, 169.0, 165.6 (J_{C-F} = 258 Hz), 139.4, 139.2, 137.3, 136.9, 136.5, 136.1, 133.2 (J_{C-F} = 3 Hz), 130.6 (2C, J_{C-F} = 9 Hz), 129.4 (2C), 128.9 (2C), 128.9 (2C), 128.7 (2C), 128.5 (2C), 128.3 (2C), 128.2 (2C), 128.0 (2C), 127.2, 126.9, 116.6 (2C, J_{C-F} = 23 Hz), 83.1, 82.2, 64.3, 58.9, 54.3, 53.3, 48.6, 35.6, 34.6, 29.4, 28.2, 23.5. Then, **22** was obtained from **22d** following the general procedure F' White solid (4.5 mg, 41%). Purity: 100%. LC t_R = 2.18 min, MS (ESI+): m/z = 568 [M + H]⁺, MS (ESI-): m/z = 566 [M - H]⁻. HRMS (ESI-TOF) calcd for C₃₀H₃₅FN₃O₅S [M + H]⁺ 568.2281, found 568.2282. Mixture of 2 amide conformers with a ratio of 1 (maj) / 0.7 (min). ¹H NMR (300 MHz, CDCl₃) δ : 7.56-7.52 (m, 3H), 7.31-7.26 (m, 2H), 7.24-7.19 (m, 5H), 7.15-7.11 (m, 4H), 7.01-6.87 (m, 4H), 6.87-6.80 (m, 3H), 5.16-5.14 (m, 1H), 4.34 (dd, J = 3.2 and 11.5 Hz, 1H), 3.75-3.63 (m, 3H), 3.57-3.44 (m, 3H), 3.27-3.12 (m, 7H), 3.05 (s, 3H), 2.95-2.88 (m, 2H), 2.67 (s, 2H), 2.07 (bs, 7H), 1.97-1.94 (m, 4H). ¹³C NMR (75MHz, CDCl₃) δ : Complex mixture of conformers. 174.2, 174.2, 171.6, 171.0, 165.4 (d, J_{C-F} = 256Hz), 139.5, 138.9, 138.3 (d, J_{C-F} = 5Hz), 136.7 (d, J_{C-F} = 7Hz), 133.3, 133.3, 130.5 (d, J_{C-F} = 9Hz), 129.2, 128.9, 128.7, 128.6, 128.4, 128.3, 128.1, 126.8, 126.7, 116.5 (d, J_{C-F} = 23 Hz), 65.1, 53.8, 53.7, 53.0, 52.8, 48.4, 48.3, 45.7, 35.9, 35.7, 34.9, 24.9, 23.5

4.1.6.3.29. (S)-2-(4-(N-(2-(diethylamino)ethyl)-4-fluorophenylsulfonamido)-N-methylbenzamido)-3-phenylpropanoic acid (**23**)

23d was obtained following the general procedure F (37 mg, 28%). LC t_R = 3.03 min, MS (ESI+): m/z = 612 [M + H]⁺, and used without further purification in the next step. **23** was obtained from **23d** following the general procedure F'. White solid (9 mg, 54%). Purity: 100%. LC t_R = 2.13 min, MS (ESI+): m/z = 556 [M + H]⁺, MS (ESI-): m/z = 554 [M - H]⁻. HRMS (ESI-TOF) calcd for C₂₉H₃₅FN₃O₅S [M + H]⁺ 556.2281, found 556.2282. Mixture of 2 conformer amides. ¹H NMR (300 MHz, CDCl₃) δ : 7.52-7.48 (m, 2H), 7.26-6.71 (m, 11H), 7.24-7.19 (m, 5H), 7.15-7.11 (m, 4H), 7.01-6.87 (m, 4H), 6.87-6.80 (m, 3H), 4.19 (dd, J = 3.4 and 11.5 Hz, 1H), 3.96-3.49 (m, 3H), 3.23-3.16 (m, 1H), 3.00-2.54 (m, 10H), 1.18-1.02 (m, 6H). ¹³C NMR (75MHz, CDCl₃) δ : Complex mixture of conformers. 176.8, 175.4, 171.9, 165.3 (d, J_{C-F} = 255Hz), 140.2, 139.2, 138.9, 138.8, 136.5 (d, J_{C-F} = 38Hz), 133.5 (d, J_{C-F} = 45Hz), 130.4 (d, J_{C-F} = 10Hz), 129.1, 128.8, 128.7, 128.6, 128.3, 128.2, 128.0, 127.8, 128.6, 128.5, 116.4 (d, J_{C-F} = 22Hz), 65.9, 51.0, 50.1, 47.9, 47.1, 46.6, 36.2, 35.8, 35.2, 29.8, 10.4

4.1.6.3.30. (S)-2-(4-(N-(2-(dimethylamino)ethyl)-4-fluorophenylsulfonamido)-N-methylbenzamido)-3-phenylpropanoic acid (**24**)

24d was obtained from general procedure E and was used without further modification in the next step. **24** was obtained from **24d** following the general procedure F. White powder (15 mg, 52%). Purity: 100%, LC t_R = 2.03 min, MS (ESI+): m/z = 528 [M + H]⁺. HRMS-ESI+ (m/z) : calcd for C₂₇H₃₁N₅O₅FS [M+H]⁺ : 528.1968; found: 528.1973. Mixture of amide conformers in a ratio of 82:18% (maj/min): ¹H NMR (300 MHz, DMSO-*d*₆) δ : 7.65-7.60 (m, 2H maj, 2H min), 7.43 (t, J = 8.3 Hz,

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2H maj, 2 H min), 7.25-7.23 (m, 3H min), 7.13-7.11 (m, 3H maj), 7.03 (d, $J = 8.4$ Hz, 2H min), 6.92 (d, $J = 8.4$ Hz, 2H min), 6.88-6.83 (m, 4H maj, 2H min), 6.73 (d, $J = 8.2$ Hz, 2H maj), 5.05 (dd, $J = 3.8$, 11.1 Hz, 1H min), 3.86 (dd, $J = 3.8$, 11.1 Hz, 1H maj), 3.69 (dd, $J = 6.7$, 13.4 Hz, 1H maj, 1H min), 3.56 (dd, $J = 6.7$, 13.4 Hz, 1H maj, 1H min), 3.14 (dd, $J = 3.8$, 14.5 Hz, 2H min), 2.76-2.67 (m, 2H maj), 2.26 (t, $J = 2.9$ Hz, 2H min), 2.19 (t, $J = 6.9$ Hz, 2H maj), 2.08 (s, 6H maj), 2.05 (s, 6H min). Complex mixture of conformers: ^{13}C NMR (75 MHz, DMSO- d_6) δ : 206.9, 204.6, 169.9 (d, $J_{\text{C-F}} = 172.0$ Hz), 141.2, 140.8, 138.7, 137.3, 133.0 (d, $J_{\text{C-F}} = 2.7$ Hz), 130.7 (d, $J_{\text{C-F}} = 8.9$ Hz), 129.2, 128.4, 128.1, 127.7, 126.1, 116.9 (d, $J_{\text{C-F}} = 22.9$ Hz), 66.6, 57.4, 48.5, 45.5, 36.4, 29.4.

4.1.6.3.31. (S)-2-(4-(4-fluoro-N-(2-(piperidin-1-yl)ethyl)phenylsulfonamido)-N-methylbenzamido)-3-phenylpropanoic acid (**25**)

25d was obtained following the general procedure E' and was used without further purification in next step. (20mg, 14%). LC $t_{\text{R}} = 3.12$ min, MS (ESI⁺): $m/z = 624$ [M + H]⁺. **25** was obtained from **25d** following the general procedure F'. White powder (10 mg, 56%). Purity: 99%, LC $t_{\text{R}} = 2.15$ min, MS (ESI⁺): $m/z = 568$ [M + H]⁺. HRMS-ESI⁺ (m/z) : calcd for C₃₀H₃₄N₃O₅FS [M+H]⁺ : 568.2281; found: 568.2286. Mixture of amide rotamers: 52:48% (maj/min). ^1H NMR (300 MHz, DMSO- d_6) δ : 10.26 (br s, 1H maj, 1H min), 7.69-7.60 (m, 2H maj, 2H min), 7.49-7.41 (m, 2H maj, 2H min), 7.33-7.28 (m, 2H maj, 2H min), 7.26-7.19 (m, 2H maj, 2H min), 7.14-7.19 (m, 1H maj, 1H min), 7.04-6.98 (m, 3H maj, 3H min), 6.58 (d, $J = 7.7$ Hz, 1H maj, 1H min), 5.16 (dd, $J = 4.7$, 11.2 Hz, 1H min), 4.32 (dd, $J = 3.8$, 10.7 Hz, 1H min), 4.06-3.83 (m, 2H maj, 2 min), 3.20-3.11 (m, 3H maj, 2H min), 3.06-2.95 (m, 3H maj, 3H min), 2.91 (m, 3H maj, 1min), 2.88-2.80 (m, 2H maj, 2H min), 2.68 (s, 3H min), 1.73-1.63 (m, 4H maj, 4H min), 1.52-1.37 (m, 2H maj, 2H min). ^{13}C NMR (75 MHz, DMSO- d_6) δ : 172.1 (maj), 171.9 (min), 171.0 (maj), 170.0 (min), 168.8 ($J_{\text{C-F}} = 240$ Hz), 139.51, 138.32, 137.82, 136.01, 133.38, 133.05, 131.22, 131.11 (d, $J_{\text{C-F}} = 11.6$ Hz, maj), 131.00 (d, $J_{\text{C-F}} = 11.6$ Hz, min), 130.24, 130.10, 129.68, 129.32, 128.76, 128.46, 127.88, 127.39, 127.08, 126.93, 117.15 (d, $J_{\text{C-F}} = 24.4$ Hz, maj), 117.0 (d, $J_{\text{C-F}} = 24.4$ Hz, maj), 63.42, 58.7, 52.95, 35.89, 34.31, 34.10, 29.51, 29.42, 23.26, 22.93, 22.10.

4.1.6.3.32. (S)-2-(4-(4-fluoro-N-(2-morpholinoethyl)phenylsulfonamido)-N-methylbenzamido)-3-phenylpropanoic acid (**26**)

26d was obtained following the general procedure E'. Yellow oil (30 mg, 25%). Purity: 89%, LC $t_{\text{R}} = 3.13$ min, MS (ESI⁺): $m/z = 627$ [M + H]⁺. Mixture of amide rotamers: 53:47% (maj/min). ^1H NMR (300MHz, CD₃OD) δ : 7.95-7.90 (m, 5H maj, 5H min), 7.68-7.64 (m, 2H maj, 2H min), 7.19-7.01 (m, 6H maj, 4H min), 6.74-6.71 (d, $J = 8.1$ Hz, 2H min), 5.05 (dd, $J = 5.2$, 5.7 Hz, 1H min), 4.37 (dd, $J = 5.2$, 5.7 Hz, 1H maj), 3.76 (t, $J = 4.7$ Hz, 2H maj, 2H min), 3.65-3.62 (m, 6H maj, 6H min), 3.40 (dd, $J = 5.6$, 14.9, 1H maj, 1H min), 3.19 (dd, $J = 5.6$, 14.9, 1H maj, 1H min), 3.00 (s, 3H maj), 2.73 (s, 3H min), 2.43-2.35 (m, 6H maj, 6H min), 1.53 (s, 9H maj, 9H min). **26** was obtained from **26d** following the general procedure F'. White powder (10 mg, 36%). Purity: 94%, LC $t_{\text{R}} = 2.08$ min, MS (ESI⁺): $m/z = 570$ [M + H]⁺. HRMS-ESI⁺ (m/z) : calcd for C₂₉H₃₃N₃O₆FS [M+H]⁺ : 570.2074; found: 570.2064. Mixture of amide rotamers: 58:42% (maj/min). ^1H NMR (300 MHz, CD₃OD) δ : 7.94-7.84 (m, 1H min), 7.69-7.60 (m, 2H maj, 1H min), 7.49-7.39 (m, 2 H maj, 2H min), 7.33-7.27 (m, 3H maj), 7.26-7.17 (m, 3H min), 7.13-7.10 (m, 2H min), 7.04-6.96 (m, 4H maj, 2H min), 6.58 (d, $J = 6.7$ Hz, 2H maj,

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2H min), 5.18-5.14 (m, 1H min), 4.33-4.30 (m, 1H maj), 3.89-3.70 (m, 1H maj, 1H min), 3.68-3.58 (m, 6H maj, 6H min), 3.20-3.08 (6H maj, 6H min), 3.07-2.94 (m, 1H maj, 1H min), 2.90 (s, 3H maj), 2.67 (s, 3H min). ^{13}C NMR (75 MHz, DMSO- d_6) δ : 183.0, 180.8, 170.4 (d, $J_{\text{C-F}} = 171.0$ Hz), 141.3, 139.2, 139.0, 138.5, 137.9, 135.9 (d, $J_{\text{C-F}} = 15.2$ Hz), 138.8, 133.0, 131.02, 131.0, 130.2, 130.0, 127.09, 127.0, 117.0 (d, $J_{\text{C-F}} = 23.5$ Hz), 116.9 (d, $J_{\text{C-F}} = 23.5$ Hz, min), 63.5, 58.8, 52.6, 52.3 (min), 35.9, 34.3 (maj), 34.0 (min), 29.5.

4.1.6.3.33. (S)-2-(4-(N-(2-(dimethylamino)ethyl)-4-fluorophenylsulfonamido)-N-methylbenzamido)-3-phenylpropanoic acid (**27**)

27d was obtained following the general procedure E'. Yellow oil (30 mg, 22%). Purity: 96%. LC $t_{\text{R}} = 2.97$ min, MS (ESI+): $m/z = 596$ [M + H] $^+$. ^1H NMR (300MHz, CDCl_3) δ : 7.69-7.66 (m, 2H), 7.63-7.56 (m, 2H), 7.32-7.26 (m, 2H), 7.21-7.11 (m, 6H), 6.62 (d, $J = 7.4$ Hz, 1H), 4.97-4.91 (m, 1H), 3.76-3.70 (m, 2H), 3.23 (d, $J = 5.8$ Hz, 1H), 2.60 (t, $J = 7.4$ Hz, 1H), 2.55-2.51 (m, 4H), 1.78-1.73 (m, 4H), 1.45 (s, 9H). ^{13}C NMR (75MHz, CDCl_3) δ : 170.8, 166.0, 165.2 ($J_{\text{C-F}} = 256$ Hz), 142.3, 136.2, 134.0 ($J_{\text{C-F}} = 3$ Hz), 133.8, 130.4 (2C, $J_{\text{C-F}} = 9$ Hz), 129.7 (2C), 128.7 (2C), 128.6 (2C), 128.0 (2C), 127.2, 116.3 (2C, $J_{\text{C-F}} = 22$ Hz), 82.9, 54.3, 54.3 (2C), 54.0, 49.6, 38.1, 28.1 (3C), 23.6 (2C). **27** was obtained from **27d** following the general procedure F'. White solid (18 mg, 66%). Purity: 100%. LC $t_{\text{R}} = 2.22$ min, MS (ESI+): $m/z = 540$ [M + H] $^+$, MS (ESI-): $m/z = 538$ [M - H] $^-$. HRMS (ESI-TOF) calcd for $\text{C}_{28}\text{H}_{31}\text{FN}_3\text{O}_5\text{S}$ [M + H] $^+$ 540.1968, found 540.1967. ^1H NMR (300 MHz, CDCl_3) δ : 7.55-7.47 (m, 4H), 7.17-6.84 (m, 9H), 4.67-4.61 (m, 1H), 3.92-3.87 (m, 2H), 3.34-3.05 (m, 8H), 2.02-1.97 (m, 4H). ^{13}C NMR (75 MHz, CDCl_3) δ : 176.3, 165.5, 165.5 ($J_{\text{C-F}} = 256$ Hz), 140.9, 138.2, 134.5, 133.0 ($J_{\text{C-F}} = 3$ Hz), 130.4 (2C, $J_{\text{C-F}} = 9$ Hz), 129.9 (2C), 128.0, 128.0 (2C), 127.5, 126.4, 116.6 (2C, $J_{\text{C-F}} = 23$ Hz), 55.3, 54.0, 53.3 (2C), 46.6, 37.6, 23.5 (4C).

4.1.6.3.34. (2S)-2-[[4-[benzenesulfonyl(2-phenylethyl)amino]benzoyl]-methyl-amino]-3-phenylpropanoic acid (**28**).

28d was obtained following the general procedure E. Yellow oil (80 mg, 67%). $t_{\text{R,LCMS}} = 8.5$ min. MS (ESI+): $m/z = 543$ [M+H- t -Bu] $^+$, and used without further purification in the next step. **28** was obtained from **28d** following the general procedure F (51 mg, 94%). LC $t_{\text{R}} = 6.7$ min. MS (ESI+): $m/z = 543$ [M+H] $^+$. ^1H NMR (300 MHz, CD_3OD) δ : Mixture of 2 cis/trans amide isomers (ratio 1/0.8) 7.68-7.58 (m, 2H), 7.57-7.42 (m, 8H), 7.38-6.92 (m, 24H), 6.87 (d, $J = 8.6$ Hz, 2H), 6.64 (d, $J = 8.2$ Hz, 2H), 5.28 (dd, $J = 5.0, 11.7$ Hz, 1H), 4.44 (dd, $J = 3.8, 11.2$ Hz, 1H), 3.86 (t, $J = 7.2$ Hz, 2H), 3.84 (t, $J = 7.1$ Hz, 2H), 3.46 (dd, $J = 5.0, 14.5$ Hz, 1H), 3.31-3.18 (m, 2H), 3.10-3.02 (m, 1H), 3.06 (s, 3H), 2.78-2.66 (m, 4H), 2.74 (s, 3H). ^{13}C NMR (75 MHz, CD_3OD) δ : 174.1, 173.4, 173.3, 172.7, 141.9, 141.6, 139.5, 139.4, 139.2 (2C), 138.8, 138.5, 136.4, 135.9, 134.2 (2C), 130.3 (2C), 130.2 (4C), 130.1 (2C), 130.0 (2C), 130.0 (2C), 129.9 (2C), 129.8 (2C), 129.8 (2C), 129.6 (2C), 129.4 (4C), 128.7 (4C), 128.3 (2C), 128.2 (2C), 128.0 (2C), 127.9 (2C), 127.5 (2C), 65.2, 60.4, 52.8, 52.8, 36.6, 36.1 (2C), 35.6, 35.3, 30.2, 18.4.

4.1.6.3.35. (2S)-2-[[4-[(4-fluorophenyl)sulfonyl(2-phenylethyl)amino]benzoyl]-methyl-amino]-3-phenylpropanoic acid (**29**).

29d was obtained following the general procedure E. Yellow oil (77 mg, 62%). $t_{\text{R,LCMS}} = 8.9$ min. MS (ESI+): $m/z = 617$ [M+H] $^+$. **29** was obtained from **29d** following the general procedure F : (32 mg, 57%). LC $t_{\text{R}} = 6.8$ min. MS (ESI+): $m/z = 561$ [M+H] $^+$. ^1H NMR (300 MHz, CD_3OD) δ : Mixture of 2

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cis/trans amide isomers (ratio 1/0.8) 7.61-7.52 (m, 4H), 7.36-6.95 (m, 28H), 6.90 (d, $J = 8.6$ Hz, 2H), 6.66 (d, $J = 8.1$ Hz, 2H), 5.28 (dd, $J = 5.0, 11.7$ Hz, 1H), 4.44 (dd, $J = 3.8, 11.1$ Hz, 1H), 3.87 (dd, $J = 7.0$ Hz, 2H), 3.85 (t, $J = 6.9$ Hz, 2H), 3.47 (dd, $J = 4.8, 14.6$ Hz, 1H), 3.29-3.19 (m, 4H), 3.12-3.03 (m, 1H), 3.07 (s, 3H), 2.78-2.69 (m, 4H), 2.75 (s, 3H). ^{13}C NMR (75 MHz, CD_3OD) δ : 174.1, 173.4 (2C), 172.7, 166.7 (d, $^1J_{\text{C-F}} = 253.3$ Hz, 2C), 141.8, 141.5, 139.5, 139.4, 138.9, 138.5, 136.6, 136.1, 135.5, 135.4, 131.6 (d, $J_{\text{C-F}} = 9.6$ Hz, 4C), 130.9 (2C), 130.8 (2C), 130.3 (2C), 130.1 (2C), 130.0 (2C), 129.9 (2C), 129.8 (4C), 129.6 (2C), 129.5 (4C), 128.4 (2C), 128.3 (2C), 128.1 (2C), 127.9 (2C), 127.5 (2C), 117.2 (d, $^2J_{\text{C-F}} = 23.0$ Hz, 4C), 65.3, 60.4, 52.8 (2C), 36.6, 36.1 (2C), 35.6, 35.3, 30.2.

4.1.6.3.36. (2S)-2-[methyl-[4-[methylsulfonyl(2-phenylethyl)amino]benzoyl]amino]-3-phenylpropanoic acid (**30**).

30d was obtained following the general procedure E. (80 mg, 100%). $t_{\text{R,LCMS}} = 7.4$ min. MS (ESI+): $m/z = 481$ [M+H-*t*-Bu] $^+$. **30** was obtained from **30d** following the general procedure F. (25 mg, 52%). LC tr = 5.7 min. MS (ESI+): $m/z = 481$ [M+H] $^+$. ^1H NMR (300 MHz, CD_3OD) δ : Mixture of 2 cis/trans amide isomers (ratio 1/0.9) 7.36-7.08 (m, 24H), 7.0-7.01 (m, 2H), 6.76 (d, $J = 8.3$ Hz, 2H), 5.29 (dd, $J = 5.0, 11.7$ Hz, 1H), 4.53 (dd, $J = 3.7, 11.2$ Hz, 1H), 3.99-3.93 (m, 4H), 3.48 (dd, $J = 5.0, 14.6$ Hz, 1H), 3.26-3.21 (m, 2H), 3.09-3.00 (m, 4H), 2.84-2.83 (m, 6H), 2.77-2.73 (m, 7H). ^{13}C NMR (75 MHz, CD_3OD) δ : 174.2, 173.4, 173.4, 172.7, 142.4, 142.1, 139.6, 139.5, 138.9, 138.5, 136.4, 135.9, 130.4 (2C), 130.1 (2C), 130.0 (2C), 130.0 (2C), 129.8 (2C), 129.8 (2C), 129.6 (2C), 129.6 (2C), 129.5 (4C), 128.7 (2C), 128.6 (2C), 128.1, 127.9, 127.6, 127.5, 65.2, 60.4, 53.0, 53.0, 37.7, 37.5, 37.4, 36.6, 36.4, 35.3, 35.6, 30.2.

4.1.6.3.37. (2S)-2-[[4-[benzenesulfonyl(2-phenylethyl)amino]benzoyl]-methyl-amino]-4-methylpentanoic acid (**31**).

31d was obtained following the general procedure E. (76 mg, 96%). LC tr = 8.5 min. MS (ESI+): $m/z = 509$ [M+H-*t*-Bu] $^+$. **31** was obtained from **31d** following the general procedure F. (30 mg, 55%). LC tr = 6.8 min. MS (ESI+): $m/z = 509$ [M+H] $^+$. ^1H NMR (300 MHz, CD_3OD) δ : Mixture of 2 cis/trans amide isomers (ratio 1/0.8) 7.69-7.60 (m, 2H), 7.60-7.46 (m, 8H), 7.42-7.31 (m, 4H), 7.26-7.06 (m, 14H), 5.27 (dd, $J = 4.9, 10.8$ Hz, 1H), 4.29 (dd, $J = 4.3, 10.8$ Hz, 2H), 4.29 (d, $J = 4.3$ Hz, 1H), 3.92 (t, $J = 7.2$ Hz, 2H), 3.91 (t, $J = 7.2$ Hz, 2H), 3.01 (s, 3H), 2.93 (s, 3H), 2.83-2.79 (m, 4H), 1.98-1.73 (m, 4H), 1.72-1.60 (m, 1H), 1.59-1.41 (m, 1.06 (d, $J = 6.8$ Hz, 1H), 1.03 (d, $J = 6.8$ Hz, 1H), 0.92 (d, $J = 6.8$ Hz, 1H), 0.70 (d, $J = 6.8$ Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ : 173.0, 172.6, 172.5, 172.4, 140.7, 138.1, 138.0, 137.8, 137.6, 135.3, 135.0, 132.8 (2C), 130.0, 128.8 (2C), 128.8 (4C), 128.8 (4C), 128.6 (4C), 128.2 (4C), 128.1 (4C), 127.3 (4C), 127.1 (2C), 126.1 (2C), 60.1, 54.9, 51.5, 51.4, 37.7, 36.8, 34.7 (2C), 33.4, 28.4, 25.0, 24.4, 22.3, 21.9, 20.5, 20.3.

4.1.6.3.38. (2S)-2-[[4-[(4-fluorophenyl)sulfonyl(2-phenylethyl)amino]benzoyl]-methyl-amino]-4-methylpentanoic acid (**32**).

32d was obtained following the general procedure E. (93 mg, 40%). $t_{\text{R,LCMS}} = 8.9$ min. MS (ESI+): $m/z = 527$ [M+H-*t*-Bu] $^+$. ^1H NMR (300 MHz, CDCl_3) δ : Mixture of 2 cis/trans amide isomers (ratio 1/0.9) 7.50-7.58 (m, 4H), 7.38-7.41 (m, 4H), 7.20-7.28 (m, 7H), 7.08-7.10 (m, 11H), 5.23-5.29 (m, 1H), 4.15-4.21 (m, 1H), 3.78 (m, 4H), 2.98 (s, 3H), 2.88 (s, 3H), 2.75-2.80 (m, 4H), 1.77-1.82 (m, 2H), 1.63-1.67 (m, 3H), 1.50 (m, 18H), 1.37-1.42 (m, 1H), 1.00-1.02 (m, 6H), 0.86 (d, $J = 6.3$ Hz, 3H), 0.64 (d, $J = 6.3$

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Hz, 3H). ^{13}C NMR (75 MHz, CDCl_3) δ : 171.7, 171.4, 170.8, 170.2, 165.2 (d, $J_{\text{C-F}} = 255$ Hz, 2C), 140.2 (2C), 137.8 (2C), 136.2, 135.9, 134.2 (d, $J_{\text{C-F}} = 3$ Hz), 134.1 (d, $J_{\text{C-F}} = 3$ Hz), 130.4 (d, $J_{\text{C-F}} = 9$ Hz, 4C), 128.9 (4C), 128.9 (2C), 128.8 (2C), 128.7 (4C), 128.1 (2C), 127.9 (2C), 126.8 (2C), 116.3 (d, $J_{\text{C-F}} = 23$ Hz, 4C), 82.6, 81.9, 60.7, 55.5, 52.2, 38.1, 37.2, 35.2 (2C), 34.0, 29.2, 28.2 (3C), 28.2 (3C), 25.5, 24.6, 23.5, 23.1, 21.7 (2C). **32** was obtained from **32d** following the general procedure F. (6 mg, 11%). LC tr = 7.0 min. MS (ESI+): $m/z = 527$ $[\text{M}+\text{H}]^+$. ^1H NMR (300 MHz, CD_3OD) δ : Mixture of 2 cis/trans amide isomers (ratio 1/0.9) 7.63-7.52 (m, 4H), 7.42-7.30 (m, 4H), 7.29-7.05 (m, 18H), 5.25 (dd, $J = 4.6, 10.4$ Hz, 1H), 4.25 (dd, $J = 5.3, 9.6$ Hz, 1H), 3.95-3.86 (m, 4H), 2.99 (s, 3H), 2.92 (s, 3H), 2.77 (m, 4H), 2.05-1.72 (m, 4H), 1.72-1.57 (m, 1H), 1.54-1.39 (m, 1H), 1.06 (d, $J = 7.0$ Hz, 1H), 1.03 (d, $J = 7.0$ Hz, 1H), 0.90 (d, $J = 6.5$ Hz, 3H), 0.68 (d, $J = 6.5$ Hz, 3H). ^{13}C NMR (75 MHz, CD_3OD) δ : 174.7 (2C), 173.9, 173.8, 166.8 (d, $J_{\text{C-F}} = 254.6$ Hz, 2C), 141.9 (2C), 139.4, 139.4, 136.9, 136.7, 135.5 (d, $J_{\text{C-F}} = 2.6$ Hz), 135.3 (d, $J_{\text{C-F}} = 2.7$ Hz), 131.6 (d, $J_{\text{C-F}} = 9.3$ Hz, 2C), 131.6 (d, $J_{\text{C-F}} = 9.7$ Hz, 2C), 130.2 (2C), 130.2 (2C), 129.9 (4C), 129.5 (4C), 1218.8 (2C), 128.6 (2C), 127.5 (2C), 117.2 (d, $J_{\text{C-F}} = 23.2$ Hz, 4C), 62.0, 56.5, 52.9, 52.8, 39.2, 38.2, 36.1 (2C), 34.8, 29.9, 26.4, 25.9, 23.7, 23.4, 21.9, 21.7.

4.1.6.3.39. (2S)-4-methyl-2-[methyl-[4-[methylsulfonyl(2-phenylethyl)amino] benzoyl]amino] pentanoic acid (**33**).

33d was obtained following the general procedure E (61 mg, 100%). $t_{\text{R,LCMS}} = 7.6$ min. MS (ESI+): $m/z = 447$ $[\text{M}+\text{H}-t\text{-Bu}]^+$. **33** was obtained from **33d** following the general procedure F (31 mg, 69%). LC tr = 5.8 min. MS (ESI+): $m/z = 447$ $[\text{M}+\text{H}]^+$. ^1H NMR (300 MHz, CD_3OD) δ : Mixture of 2 cis/trans amide isomers (ratio 1/0.7) 7.53-7.41 (m, 8H), 7.33-7.10 (m, 10H), 5.29 (dd, $J = 5.3, 10.7$ Hz, 1H), 4.36 (dd, $J = 5.0, 9.6$ Hz, 1H), 4.07-3.98 (m, 4H), 3.02 (s, 3H), 2.96 (s, 3H), 2.89 (s, 3H), 2.88 (s, 3H), 2.86-2.77 (m, 4H), 2.01-1.75 (m, 4H), 1.74-1.65 (m, 1H), 1.57-1.41 (m, 1H), 1.07 (d, $J = 6.9$ Hz, 3H), 1.04 (d, $J = 6.5$ Hz, 3H), 0.91 (d, $J = 6.7$ Hz, 3H), 0.70 (d, $J = 6.5$ Hz, 3H). ^{13}C NMR (75MHz, CD_3OD) δ : 173.0, 172.6, 172.5 (2C), 141.2, 141.1, 138.2, 138.1, 135.2, 134.9, 130.4, 128.6 (4C), 128.5 (2C), 128.1 (4C), 127.7, 127.6 (2C), 127.4 (2C), 126.2 (2C), 60.2, 54.9, 51.6, 51.6, 37.6, 36.8, 36.1, 36.1, 35.0 (2C), 33.4, 28.5, 25.0, 24.4, 22.3, 21.9, 20.5, 20.3.

4.1.6.3.40. *Synthesis of 4-(4-fluoro-N-(2-(pyrrolidin-1-yl)ethyl)phenylsulfonamido)benzoic acid (17a).*

A solution of 4-fluorobenzenesulfonyl chloride (1.11 g, 5.69 mmol, 1.1 eq.) and tert-butyl 4-aminobenzoate (1.0 g, 5.17 mmol) in pyridine (5mL) was stirred for 3 hours at 70°C. The mixture was concentrated under reduced pressure. Water was added to the residue, and the aqueous layer was extracted three times with CH_2Cl_2 . The organic layers were gathered, washed with 1N HCl, water, and brine, dried over MgSO_4 , filtered, and concentrated under reduced pressure to afford tert-butyl 4-(4-fluorophenylsulfonamido)benzoate as a yellowish oil (1.77 g, 97%). LC tr: 3.17min, MS (ESI-): $m/z = 350$ $[\text{M}-\text{H}]^-$. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ : 10.81 (s, 1H), 7.90-7.84 (m, 2H), 7.79-7.74 (m, 2H), 7.45-7.30 (m, 2H), 7.22-7.17 (m, 2H), 1.49 (s, 9H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ : 164.3 (d, $J = 252.8\text{Hz}$), 164.2, 141.6, 135.4 (d, $J = 3.0\text{Hz}$), 130.4 (2C), 129.7 (d, $J = 9.6\text{Hz}$, 2C), 126.3, 118.3 (2C), 116.5 (d, $J = 22.8\text{Hz}$, 2C), 80.4, 27.6 (3C). Then, 1-(2-chloroethyl)pyrrolidine hydrochloride (1.59 g, 9.33 mmol, 2 eq.) was added to a solution of tert-butyl 4-[(4-fluorophenyl)sulfonylamino]benzoate (1.64 mg, 4.67 mmol, 1 eq.) and K_2CO_3 (1.94 g, 14.0 mmol, 3 eq.) in DMF (5 mL), previously stirred for 10 min, and

[Tapez ici]

the mixture was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure, and water was added to the residue. The aqueous layer was extracted three times with EtOAc. The organic layers were gathered together, washed with water, then with brine, dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (cyclohexane/EtOAc 100:0 to 80:20 (v/v)) to give tert-butyl 4-(4-fluoro-N-(2-(pyrrolidin-1-yl)ethyl)phenylsulfonamido)benzoate as a yellow oil (450 mg, 22%). Purity: 86%, LC t_R = 2.83 min, MS (ESI⁺): m/z = 449 [M + H]⁺. ¹H NMR (300 MHz, CD₃OD) δ: 7.92 (d, *J* = 8.4 Hz, 2H), 7.63 (m, 2H), 7.31-7.21 (m, 4H), 3.79 (t, *J* = 7.1 Hz, 2H), 2.58 (t, *J* = 7.1 Hz, 2H), 2.56-2.51 (m, 4H), 1.79-1.75 (m, 4H), 1.59 (s, 9H). ¹³C NMR (75 MHz, CD₃OD) δ: 166.3, 165.9 (d, *J*_{C-F} = 237.6 Hz), 144.4, 132.3 (m),, 132.6, 131.7 (d, *J*_{C-F} = 9.3 Hz), 131.6, 129.5, 117.4 (d, *J*_{C-F} = 23.3 Hz), 82.7, 55.3, 55.0, 50.3, 28.3, 24.2. HCl (4N in dioxane, 3 mL) was added to a solution of tert-butyl 4-[(4-fluorophenyl)sulfonyl-(2-pyrrolidin-1-ylethyl)amino]benzoate (450 mg, 1 mmol, 1 eq.) in CH₂Cl₂ (3 mL), and it was stirred overnight at rt. The solid in the mixture was recovered by filtration, and washed with diethyl ether. **17a** was obtained as a white powder (410 mg, 95%). Purity: 98%, LC t_R = 1.87 min, MS (ESI⁺): m/z = 393 [M + H]⁺. ¹H NMR (300 MHz, DMSO-*d*₆) δ: 11.11 (br s, 1H), 7.93 (d, *J* = 8.6 Hz, 2H), 7.64 (dd, *J* = 5.3, 8.9 Hz, 2H), 7.46 (t, *J* = 8.9 Hz, 2H), 7.31 (d, *J* = 8.6 Hz, 2H), 4.04 (t, *J* = 7.1 Hz, 2H), 3.54-3.48 (m, 2H), 3.24-3.17 (m, 2H), 3.03-2.90 (m, 2H), 1.96-1.80 (m, 4H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ: 166.5, 164.8 (d, *J*_{C-F} = 248.5 Hz), 142.0, 132.7 (m),, 130.7 (d, *J*_{C-F} = 9.4 Hz, 2CH), 130.4, 130.2 (2CH), 128.2 (2CH), 116.8 (d, *J*_{C-F} = 22.7 Hz, 2CH), 53.0, 51.3, 45.9, 22.6.

4.1.6.3.41. Synthesis of (*R*)-tert-butyl 2-(methylamino)-3-phenylpropanoate (**17b**)

2-nitrobenzenesulfonyl chloride (1.12 g, 5.04 mmol, 1.3 eq.) was added in portions to a solution of tert-butyl (2*R*)-2-amino-3-phenylpropanoate hydrochloride (1 g, 3.8 mmol, 1 eq.), and K₂CO₃ (1.07 g, 7.7 mmol, 2 eq.) in DMF (30 mL) at 0°C under argon, and the mixture was stirred at room temperature overnight. The reaction mixture was concentrated under reduced pressure, and ethyl acetate was added to the residue. The organic layer was washed three times with water, and the final aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with water, then with brine, dried over MgSO₄, filtered, and concentrated under reduced pressure to give (*R*)-tert-butyl 2-(2-nitrophenylsulfonamido)-3-phenylpropanoate (1.5 g, 89%). Purity: 94%, LC t_R = 3.2 min, MS (ESI⁻): m/z = 405 [M - H]⁻. ¹H NMR (300 MHz, CD₃OD) δ: 7.90-7.88 (m, 1H), 7.81-7.65 (m, 3H), 7.17 (s, 5H), 4.21 (dd, *J* = 6.2, 8.4 Hz, 1H), 3.07 (dd, *J* = 6.2, 13.6 Hz, 1H), 2.93 (dd, *J* = 8.4, 13.6 Hz, 1H), 1.24 (s, 9H). ¹³C NMR (75 MHz, CD₃OD) δ: 171.5, 149.2, 137.5, 135.3, 134.8, 133.7, 131.5, 130.5, 129.4, 128.0, 125.9, 83.2, 59.9, 39.9, 28.0. Iodomethane (1.04 mL, 16.7 mmol, 4eq.) was added dropwise to a solution of tert-butyl (2*R*)-2-[(2-nitrophenyl)sulfonylamino]-3-phenylpropanoate (1.7 g, 4.18 mmol, 1 eq.), and K₂CO₃ (1.16 g, 8.4 mmol, 2 eq.) in DMF (10 mL) at 0°C under argon, previously stirred for 10 min. The reaction mixture was stirred overnight at room temperature. The reaction was quenched by addition of saturated aqueous NH₄Cl. The mixture was extracted three times with ethyl acetate, and the combined organic extracts were washed with HCl, then with brine, dried (MgSO₄), filtered, and concentrated under reduced pressure. (*R*)-tert-butyl 2-(*N*-methyl-2-nitrophenylsulfonamido)-3-phenylpropanoate was obtained as a yellow oil. (1.56 g, 84%). Purity: 95%,

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LC t_R = 3.32 min, MS (ESI⁺): m/z = 438 [M + NH₄⁺]⁺. ¹H NMR (300 MHz, CD₃OD) δ : 7.78-7.72 (m, 2H), 7.69-7.62 (m, 2H), 7.24-7.16 (m, 5H), 4.82 (dd, J = 6.3, 9.6 Hz, 1H), 3.27 (dd, J = 6.3, 14.7 Hz, 1H), 3.02 (s, 3H), 2.98 (dd, J = 9.6, 14.7 Hz, 1H), 1.31 (s, 9H). ¹³C NMR (75 MHz, CD₃OD) δ : 170.5, 138.0 (2C), 135.0, 133.6, 133.0, 131.8, 130.3, 129.5, 127.9, 125.3, 83.4, 62.9, 36.6, 31.3, 28.1. Tert-butyl (2*R*)-2-[methyl-(2-nitrophenyl)sulfonyl-amino]-3-phenyl-propanoate (1.7 g, 4.04 mmol, 1 eq.) was dissolved in DMF (10 mL) at 0°C and Cs₂CO₃ (1.32 mg, 4.0 mmol, 1 eq.) and LiOH/H₂O (170 mg, 4.0 mmol, 1 eq.) were added, followed by mercaptoacetic acid (1128 μ L, 16.2 mmol, 4 eq.). The mixture was stirred at 60 °C overnight, then concentrated under reduced pressure, and ethyl acetate was added to the residue. The organic layer was washed three times with water, and the final aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with water, then with brine, dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (cyclohexane/EtOAc 100:0 to 80:20 (v/v)) to give the desired product as a yellow oil (210 mg, 22%). Purity: 44%, LC t_R = 2.21 min, MS (ESI⁺): m/z = 180 [M - *t*-Bu]⁺, 236 [M + H]⁺. ¹H NMR (300 MHz, CD₃OD) δ : 7.31-7.19 (m, 5H), 3.93 (dd, J = 2.3, 4.6 Hz), 2.97 (dd, J = 4.6, 13.2 Hz, 1H), 2.84 (dd, J = 2.3, 13.2 Hz, 1H), 2.34 (s, 3H), 1.32 (s, 9H). ¹³C NMR (75 MHz, CD₃OD) δ : 174.4, 135.0, 130.5, 129.4, 127.7, 82.5, 66.3, 40.2, 34.3, 28.2.

4.1.6.3.42. Synthesis of (*R*)-2-(4-(4-fluoro-*N*-(2-(pyrrolidin-1-yl)ethyl)phenyl)sulfonamido)benzamido)-3-phenylpropanoic acid (**17**)

A mixture of tert-butyl (2*R*)-2-(methylamino)-3-phenyl-propanoate **17b** (65.9 mg, 0.28 mmol, 1.2 eq.), triethylamine (71.1 μ L, 0.51 mmol, 2.2 eq.), HOBt (19.5 mg, 0.13 mmol, 0.5 eq.), HBTU (145 mg, 0.38 mmol, 1.6 eq.), and 4-[(4-fluorophenyl)sulfonyl-(2-pyrrolidin-1-ylethyl)amino]benzoic acid **17a** (100 mg, 0.23 mmol, 1 eq.) in DMF (3 mL) was stirred overnight at room temperature. Water was added to the solution and the resulting mixture was extracted with 3 times with ethyl acetate. The resulting organic layer was washed with aq. sat. NaHCO₃ solution, aq. HCl (0.1 M) and brine. The organic phase was dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue containing the desired product was purified by flash chromatography on silica gel (cyclohexane/EtOAc 100:0 to 90:10) to give tert-butyl (2*S*)-2-[(4-iodobenzoyl)amino]-3-phenyl-propanoate. Purity: 73%. LC t_R = 3.03 min, MS (ESI⁺): m/z = 611 [M + H]⁺. Mixture of amide isomers (conformers), approximate ratio : 0.53/0.47 (1.1/1). ¹H NMR (300 MHz, CD₃OD) δ : 7.67-7.59 (m, 2H), 7.35-7.23 (m, 6H), 7.18 (br d, J = 8.4Hz, 1H), 7.09 (br d, J = 8.4Hz, 1H), 7.07-7.00 (m, 2H), 6.72 (br d, J = 8.1Hz, 1H), 5.05 (dd, J = 11.1, 5.3Hz, 0.5H), 4.38 (dd, J = 10.6, 4.2Hz, 0.5H), 3.88-3.76 (m, 2H), 3.39 (dd, J = 14.3, 5.3Hz, 0.5H), 3.23 (dd, J = 14.3, 11.1Hz, 0.5H), 3.17 (dd, J = 14.1, 4.2Hz, 0.5H), 3.05 (s, 1.5H), 3.01 (dd, J = 14.1, 10.6Hz, 0.5H), 2.85-2.67 (m, 7.5H), 1.92-1.79 (m, 4H), 1.52 (s, 9H). ¹³C NMR (75MHz, CD₃OD) δ : major isomer: 174.0, 170.2, 166.9 (d, J = 254.1Hz), 141.3, 138.4, 137.0, 135.0 (d, J = 3.0Hz), 131.8 (d, J = 9.6Hz, 2C), 130.4 (2C), 129.8 (2C), 129.7 (2C), 128.5 (2C), 128.1, 117.4 (d, J = 23.5Hz, 2C), 84.0, 65.8, 55.2 (2C), 55.1, 49.8, 35.3, 30.0, 28.2 (3C), 24.1 (2C); minor isomer: 173.0, 170.8, 166.9 (d, J = 254.1Hz), 141.6, 138.9, 136.5, 135.0 (d, J = 3.0Hz), 131.8 (d, J = 9.6Hz, 2C), 130.1 (2C), 130.0 (2C), 129.6 (2C), 128.6 (2C), 127.8, 117.4 (d, J = 23.5Hz, 2C), 83.2, 61.8, 55.2 (2C), 55.0, 49.8, 37.1, 35.4, 28.3 (3C), 24.1 (2C). HCl (4N in dioxane, 3 mL) was added to a solution of tert-butyl 4-[(4-

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fluorophenyl)sulfonyl-(2-pyrrolidin-1-ylethyl)amino]benzoate (400 mg, 0.66 mmol, 1 eq.) in CH₂Cl₂ (3 mL), and it was stirred overnight at rt.. The solid in the mixture was recovered by filtration, and washed with diethyl ether. The desired product was obtained as a white powder. LC tr = 2.05 min. MS (ESI+): m/z = 554 [M+H]⁺. ¹H NMR (300MHz, DMSO-*d*₆) δ: Mixture of 2 cis/trans amide isomers: 7.68-7.62 (m, 2H), 7.43 (t, *J* = 8.6 Hz, 1.2H), 7.41 (t, *J* = 8.7 Hz, 0.8H), 7.34-7.14 (m, 4H), 7.11 (d, *J* = 8.3 Hz, 0.8H), 7.01 (d, *J* = 8.3 Hz, 0.8H), 7.00-6.94 (m, 2.4H), 6.65 (br d, *J* = 8.0 Hz, 1H), 5.16 (dd, *J* = 11.2, 4.8 Hz, 0.4H), 4.22 (dd, *J* = 10.7, 3.6 Hz, 0.6H), 3.83-3.62 (m, 2H), 3.32 (dd, *J* = 14.5, 4.9Hz, 0.4H), 3.16-3.08 (m, 1H), 2.96-2.88 (m, 0.6H), 2.89 (s, 1.7H), 2.67 (s, 1.3H), 2.60-2.51 (m, 6H), 1.71-1.63 (m, 4H). ¹³C NMR (75 MHz, DMSO-*d*₆) δ: major isomer: 171.8, 170.4, 164.6 (d, *J* = 252.1Hz), 138.8, 138.1, 135.6, 133.8 (d, *J* = 3.0Hz), 130.3 (d, *J* = 9.6Hz, 2C), 129.0 (2C), 128.2 (2C), 128.2 (2C), 126.9 (2C), 126.3, 116.4 (d, *J* = 22.7Hz, 2C), 63.8, 53.2 (2C), 53.1, 48.5, 34.4, 28.9, 23.0 (2C). Minor isomer: 171.7, 169.8, 164.6 (d, *J* = 252.1Hz), 139.4, 138.1, 135.6, 133.8 (d, *J* = 3.0Hz), 130.3 (d, *J* = 9.6Hz, 2C), 129.0 (2C), 128.2 (2C), 128.2 (2C), 126.9 (2C), 126.3, 116.4 (d, *J* = 22.7Hz, 2C), 58.3, 53.2 (2C), 53.1, 48.5, 35.1, 33.8, 23.0 (2C).

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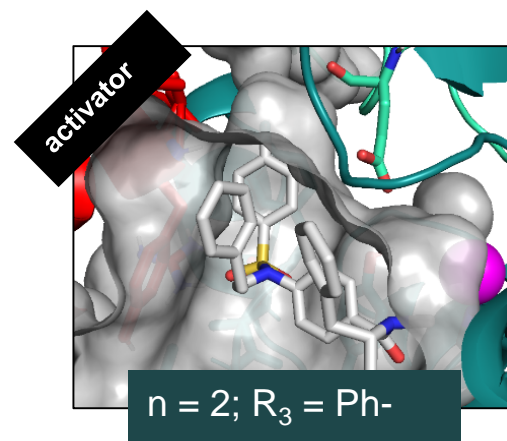
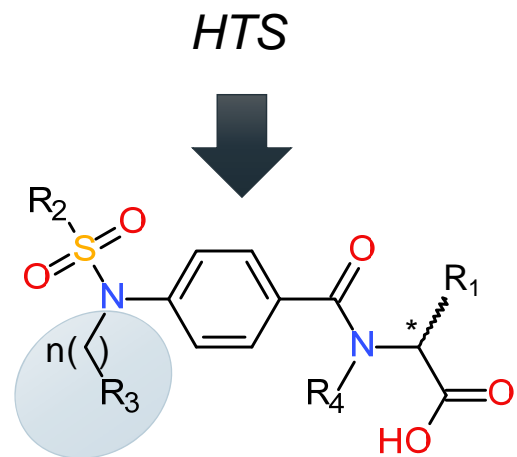
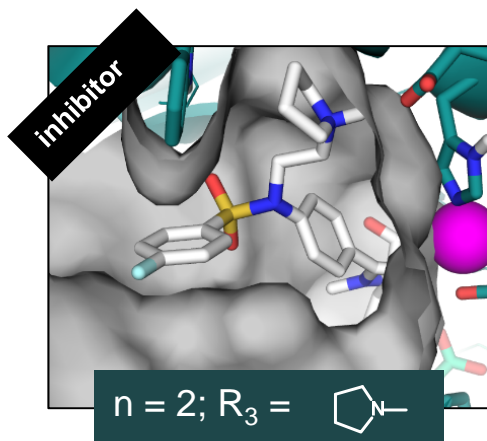
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