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► **To cite this version:**

Nicolas Kraupner, Chau Phi Dinh, Xiaoan Wen, Valérie Landry, Adrien Herledan, et al.. Identification of indole-based activators of insulin degrading enzyme. *European Journal of Medicinal Chemistry*, 2021, pp.113982. 10.1016/j.ejmech.2021.113982 . hal-03430475

HAL Id: hal-03430475

<https://hal.science/hal-03430475>

Submitted on 8 Jan 2024

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Identification of indole-based activators of Insulin Degrading Enzyme[†]

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Abstract

Insulin degrading enzyme (IDE) is a zinc metalloprotease that cleaves numerous substrates among which amyloid- β and insulin. It has been linked through genetic studies to the risk of type-2 diabetes (T2D) or Alzheimer's disease (AD). Pharmacological activation of IDE is an attractive therapeutic strategy in AD. While IDE inhibition gave paradoxical activity in glucose homeostasis, recent studies, in particular in the liver suggest that IDE activators could be also of interest in diabetes. Here we describe the discovery of an original series of IDE activators by screening and structure-activity relationships. Early cellular studies show that hit **1** decreases glucose-stimulating insulin secretion. Docking studies revealed it has an unprecedented extended binding to the polyanion-binding site of IDE. These indole-based pharmacological tools are activators of both A β and insulin hydrolysis by IDE and could be helpful to explore the multiple roles of IDE.

Keywords

Insulin-degrading enzyme; activators; screening; metalloenzymes

[†] **Abbreviations:** AD, Alzheimer's disease; APP, amyloid precursor-peptide; EDTA, ethylene-diamino-tetra-Acetate; IDE, insulin degrading enzyme; IGF-II, insulin growth factor -2; T2D, type-2 diabetes, T3P®, propylphosphonic anhydride; TGF- α , transforming growth factor α .

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Introduction

Insulin-Degrading Enzyme (IDE) is a Zinc endopeptidase of the M16 family involved in the degradation of many substrates. In particular, it is the main enzyme responsible for the intracellular hydrolysis of insulin, the most affine substrate for IDE [1]. Glucagon [2] and amylin [3], two other peptidic hormones secreted by the pancreas, are also substrates. IDE was subsequently proposed as one of the enzymes involved in the catabolism of the amyloid peptide (A β) [4]. Finally, the calcitonin gene-related peptide CGRP and many other peptides with various length and affinities like somatostatin, IGF-II, TGF- α , β -endorphin, dynorphins, bradykinin, atrial natriuretic peptide (ANP) are substrates of IDE [5]. The reason underlying the ubiquitous expression of IDE is unknown. Still the diversity of the substrates and the non-proteolytic roles of IDE could explain this large distribution of IDE. For example, IDE is involved in the ubiquitin-proteasome pathway since the monomeric ubiquitin is degraded by IDE [6] and since IDE directly regulates the activity of the proteasome by protein-protein interaction [7]. Interestingly, many substrates of IDE share a propensity to be amyloidogenic peptides [8] and IDE can be seen as a protein that prevents the formation of toxic peptide oligomers [9]. This possibly conserved function could explain the high level of sequence homology across distantly related species. Interestingly an enzymatically inactive variant of IDE conserves this function *in vitro* [10]. Moreover, the fission yeast IDE homologue named *lph1* was shown to modulate the sensitivity of cells to endoplasmic reticulum (ER) stress in a manner dependent on TORC1 and independent of its catalytic activity [11].

Along with the biochemical activity of peptide hydrolysis, genetic and epidemiological studies on populations associated a polymorphism within IDE gene loci to the risk of type 2 diabetes (T2D) [12]. The role of IDE in insulin levels and glucose tolerance has attracted a lot of attention in the past decade [13], [14]. Using both cellular assays or animals with pan cellular genetic *Ide* deletion, tissue selective genetic *Ide* deletion [15], [16], *Ide* overexpression or pharmacological inhibition [17,18,19] several teams including us have shown the complex role of IDE in glucose tolerance far beyond the hydrolysis of insulin, in particular in organs involved in the control glucose levels like pancreas and liver.

Variants of the *ide* gene and loci of the IDE gene on chromosome 10q have also been associated with plasma concentrations of A β 1-42 and increased risk for Alzheimer's disease (AD) [20], [21]. The role of clearance of A β by IDE has been demonstrated using IDE *-/-* mice, [22] or transgenic mice overexpressing both APP and IDE [23].

IDE is a dynamic protein whose structure consists in an N-terminal part (IDE N), encompassing domains 1 and 2 (residues 43-285 and 286-515 respectively) and a C-terminal part (IDE C), consisting of domains 3 and 4 (residues 542-768 and 769-1016 respectively articulated around a flexible loop [24] (**Fig. 1**). It exists in solution in equilibrium between an open form and a closed form. Several biophysical studies have captured the displacement of a subdomain that creates an opening to the catalytic chamber for the substrates [25]. The dynamics of the subdomain displacement has been shown to be disrupted by inhibitors thus impacting IDE's catalytic activity [26]. In addition to the catalytic pocket containing the Zinc ion and HXXEH and GAMEN sequences, IDE displays an exosite located ~ 30 Å away from the catalytic pocket, in the domain 2 (**Fig. 1**). This exosite has been shown to allow anchoring of the N-terminus of substrates causing a rearrangement of their secondary structure in order to facilitate binding and subsequent cleavages at the catalytic site [24]. The co-crystallizations of the IDE with ATP showed also an ATP pocket at domain 4 (IDE-C) of the protein [27] (**Fig. 1**).

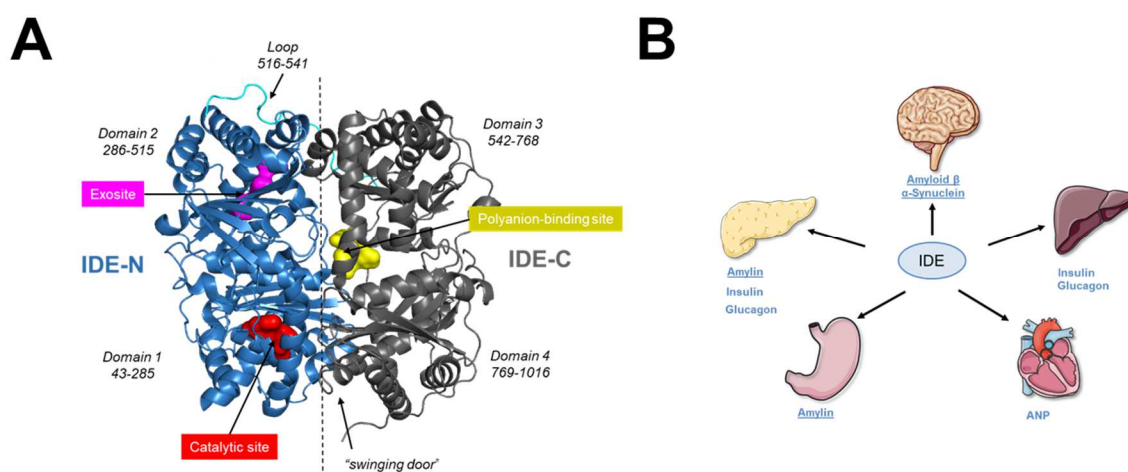


Figure 1: Structure of IDE and key substrates^a

^a (A) Structure of hIDE (from PDB 4NXO); IDE- N and IDE-C halves are colored in blue and gray respectively. Catalytic residues, exosite residues and ATP-binding residues are colored in red, magenta and yellow respectively. The “swinging door” and the loop are pointed with an arrow. (B) Main substrates of IDE and targeted organs. Underlined are peptides that can oligomerize.

Comforted by the disease implications of IDE, and in particular in T2D, several teams have in the past decade developed inhibitors of IDE via rational design [28], high-throughput screening [19,29-30], zinc-binding fragment screening [31], DNA-templated library screening [18], drug repurposing [32] or kinetic target-guided synthesis [17]. The inhibitors were shown to bind the catalytic site [17,28-29] or exosites [29,18,19]. Some are pan-IDE inhibitors [17, 28], others are substrate selective inhibitors [33]

and some others are inhibitors of the hydrolysis of one substrate and activator of the hydrolysis of others [29,30].

Inhibitors have produced some controversial results regarding insulin clearance and their application as modulators of glucose tolerance is at stake. Still, they are highly useful tools to decipher the atypical roles of IDE.

Activators are also foreseen. First, there is a need for compounds that act as activators with respect to A β degradation, in order to decrease the levels of this toxic peptide in AD. Secondly, regarding insulin clearance and glucose homeostasis, recent studies have underlined the complex role of IDE in both pancreas and insulin sensitive tissues like liver [14-15]. As a consequence, activators could be worth exploring for glucose homeostasis too [34]. However, in contrast to inhibitors, only a handful of activators of this enzyme have been disclosed (Fig. 2). Suramin, was the first identified activator of the hydrolysis of the peptide A β by IDE [35] (Fig. 2). Unfortunately, suramin is non-permeable across the cell membrane, toxic and not suitable for *in vivo* studies. In 2009, activators **la1** and **la2** were discovered by screening [36] (Fig. 2). A series of polyaromatic compounds **D3**, **D4**, **D6** and **D10** (Fig. 2) were identified through a virtual screening at the exosite. These compounds displayed IDE activation of insulin degradation over other substrates [37]. Selective activators of rat IDE over human IDE, were discovered by high-throughput screening and displayed cellular activity (**rat-7**, Fig. 2) [38]. We also described a series of small molecules showing a dual modulation of IDE. By binding at two different sites, the catalytic site and the exosite, these compounds, like **BDM43079** are inhibitors of amyloid degradation and activators of the hydrolysis of insulin and IGF-II [39] (**Fig. 2**).

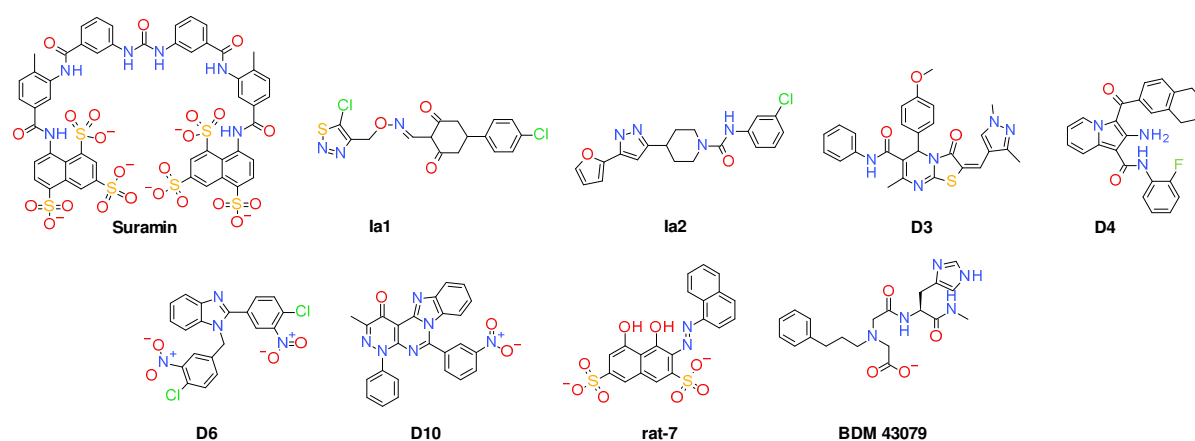


Figure 2: Activators of IDE from the literature [35-39].

Here we describe the screening of a collection of more than 8 000 compounds and the discovery of indole-based activators of IDE. A set of 50 analogues allowed the establishment of structure-activity relationships and study of binding via docking. The best compound was shown to be activator of both A β and insulin hydrolysis by IDE and displayed cellular activity in a glucose-stimulated insulin secretion assay.

1. Results and Discussion

1.1. Screening of an 8500-member library.

With the aim of discovering new activators to explore the complex role of IDE, we screened at 30 μ M a library of 8 500 drug-like compounds from *in-house* and commercial sources. Figure 3 summarizes the screening cascade. The compounds were screened in a fluorescence assay monitoring the hydrolysis by IDE of an A β substrate analogue (Supporting information Figure S1). Suramin at 10 μ M was used as a positive control and displayed stable activity throughout the screening (Supporting information Figure S2).

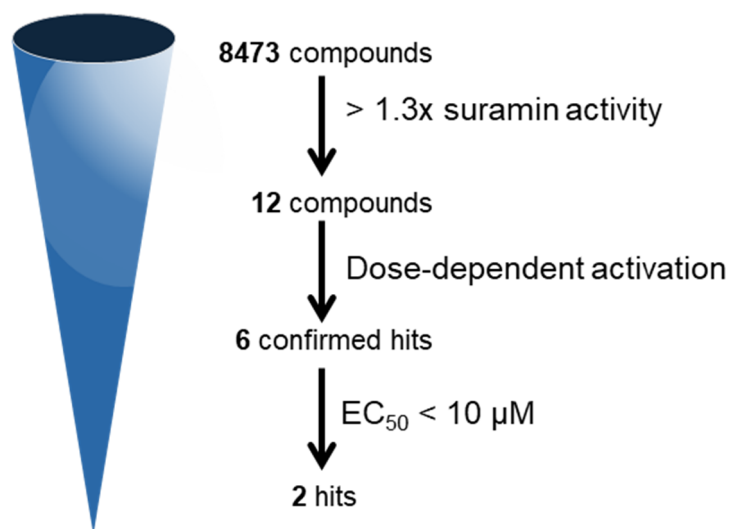


Figure 3: Screening cascade^a

The screening allowed the identification of 12 activators (0.14% hit rate) that displayed enzyme activity of at least 1.3 times that observed in the presence of 10 μ M suramin (Fig. 3 and Supplementary Table S1 and Figures S3-S4).

Dose-response curves were established and among the 6 confirmed hits, 2 displayed an EC₅₀ below 10 μ M. Among them, indole derivative **1** with a potency (EC₅₀) of 5.5 μ M, an activation efficacy of

233% (2.33 fold) at 100 μ M and a promising lipophilic ligand efficiency LLE of 2.02, was selected for further pharmacomodulation (**Fig. 4**, Supplementary Figure S5).

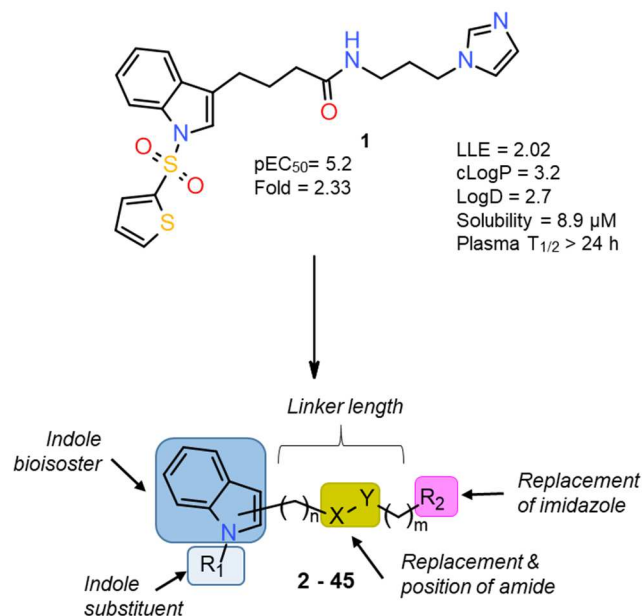


Figure 4: Structure of hit **1** and planned pharmacomodulation.

1.2. Biological characterization of hit **1**.

We first measured some *in vitro* ADME properties of hit **1**. Hit **1** displays a medium LogD (2.7 at pH 7.4), a moderate aqueous solubility (8.9 μ M) and a very good plasmatic stability (Half-life in rat plasma > 24 h) (Fig.4). We then evaluated the behavior of **1** regarding the hydrolysis of a second substrate of IDE: insulin (Fig. 5 and Supplementary Table S2). EDTA was used as a control inhibitor. **1** behaves as an activator of full-length insulin hydrolysis by IDE.

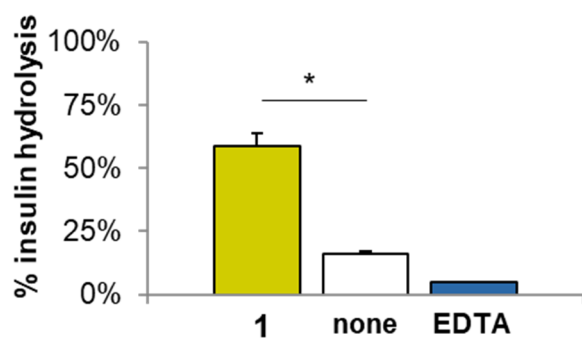


Figure 5: Hydrolysis of insulin by IDE in the presence of cpd **1**.^a

^a Values are given as the mean of 3 experiments. [hIDE] = 7.5 μ g/mL. [**1**] = 100 μ M; [EDTA] = 50 mM. EDTA inhibits IDE by chelating the zinc, whereas **1** behaves as an activator of insulin hydrolysis by IDE. * $p < 0.01$.

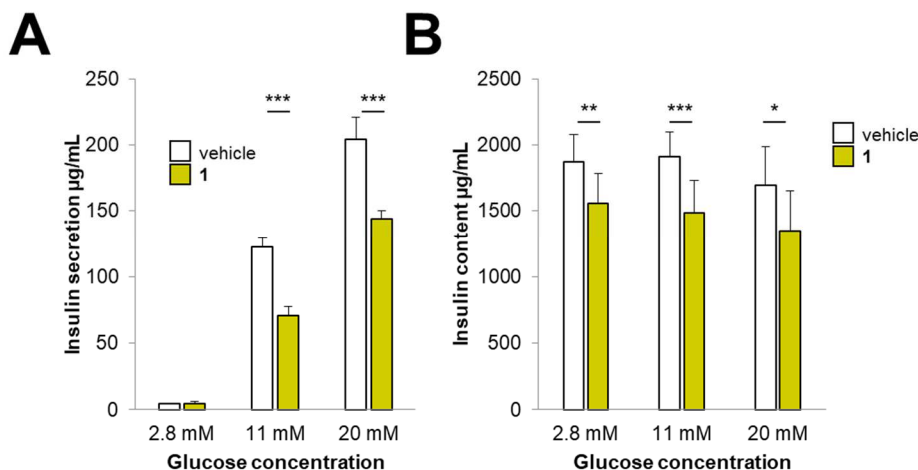


Figure 6: Effect of **1** on the glucose stimulation of insulin secretion (GSIS) on Min6 β -pancreatic cells.^a
^a A) Insulin secretion induced by glucose stimulation in the presence of **1**, overnight pretreatment before and during GSIS. B) Insulin content in the presence of increasing glucose concentrations, and in the presence of **1**. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Values are given as the mean of 3 experiments.

In an assay of insulin secretion induced by glucose by pancreatic β -cells (Fig. 6), **1** decreases insulin secretion significantly for medium and high glucose stimulation (Fig. 6A). In the meantime, insulin content is decreased in the presence of **1**, compared to vehicle treatment (Fig. 6B).

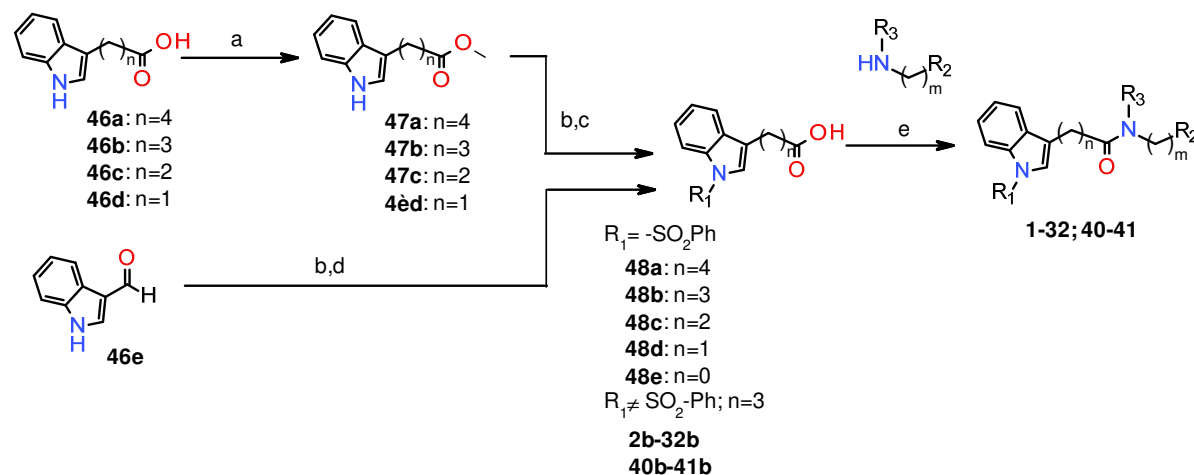
1.3. Synthesis of analogues.

To explore structure-activity relationships around hit **1**, we synthesized 47 analogues using 8 synthetic routes (Schemes 1-8). Analogues **2-25** were synthesized to investigate the impact of the N-substitution on the indole. Analogues **26-39** allowed the exploration of the linker length, branching or truncation as well as the positioning of the amide group. Finally, analogues **40-45** aimed at evaluating the impact of both indole and imidazole isosteric modifications.

Compounds **1-32** and **40-41** were obtained as described in Scheme 1. Commercial 3-carboxyalkyl-1*H*-indole precursors (**46a-d**) were esterified in methanol and H_2SO_4 at room temperature. N-alkylation, N-sulfonylation or N-acylation of the indole nitrogen was performed in the presence of NaOH as a base, and catalytic amount of Bu_4NHSO_4 , starting from the corresponding halides, acid chlorides or sulfonylhalides and afforded N-protected indoles **48a-e**, **2b-32b**, **40b-41b**. The methyl esters were then saponified into carboxylic acids, which were then activated using HOBt/EDCI and reacted with amines in DMF in the presence of DIPEA to afford the desired amides (**1-25**, **27-32**, **40-41**).

To obtain analogue **26**, the commercial indolebutyric aldehyde (**46e**) reacted first with benzenesulfonylchloride to give the N-sulfonylated indole intermediate and then oxidized to the

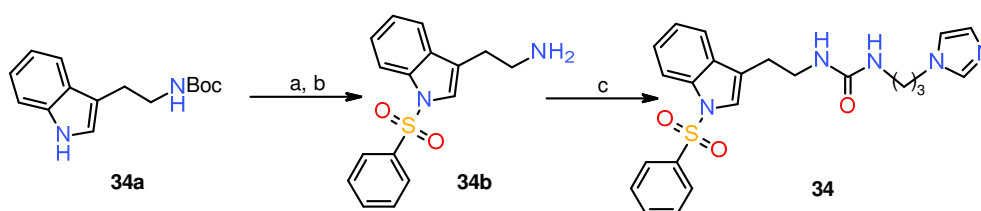
carboxylic acid **48e** using sodium chlorite and sulfamic acid. **48e** was then reacted with 1-(3-aminopropyl)-imidazole to give **26** as previously described (Scheme 1).



Scheme 1. Synthesis of indole derivatives **1-32** and **40-41**.^a

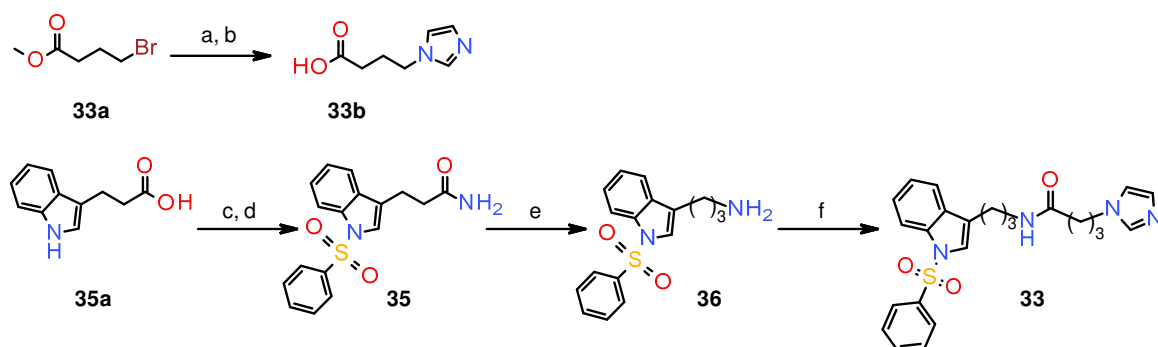
^a *Reactants and conditions:* a) H_2SO_4 , MeOH, room temp., 2 h, 98%; b) NaOH, Bu_4NHSO_4 cat., DCM, halide, sulfonylchloride or acylhalide, room temp., 3 h or overnight; c) NaOH, MeOH/ H_2O , room temp., overnight, 27%-quant (2-step yield); d) NaClO_2 , HOSO_2NH_2 , dioxane/ H_2O , room temp., 1 h, 56% (2-step yield); e) HOBT/EDCI, DIPEA, DMF, room temp., overnight, 18-88%.

Urea analogue **34** was obtained in 3 steps from the N-Boc-tryptamine (**34a**) (Scheme 2). **34a** was substituted with benzenesulfonyl chloride using NaOH and Bu_4NHSO_4 (cat.) before the amine was deprotected using HCl 4N in dioxane. Amine **34b** reacted with 1-(3-aminopropyl)-imidazole in the presence of 4-nitrophenyl chloroformate and trimethylamine in THF to give urea **34** (Scheme 2). Retro-amide **33** required the synthesis of 4-imidazol-1-ylbutanoic acid **33b** from commercial methyl 4-bromobutanoate (Scheme 3). The commercial indolebutyric acid precursor (**35a**) reacted with benzenesulfonyl chloride, then the carboxylic acid was turned into the corresponding propanamide **35** with HOBT, EDCI and NH_4Cl . Then amide group was reduced by NaBH_4 and AlCl_3 to give the amine **36**, which was coupled with previously synthesized carboxylic acid **33b** to afford amide **33** (Scheme 3). Both truncated analogues **35** and **36** were kept for biological testing.



Scheme 2. Synthesis of the indole derivative **34**.^a

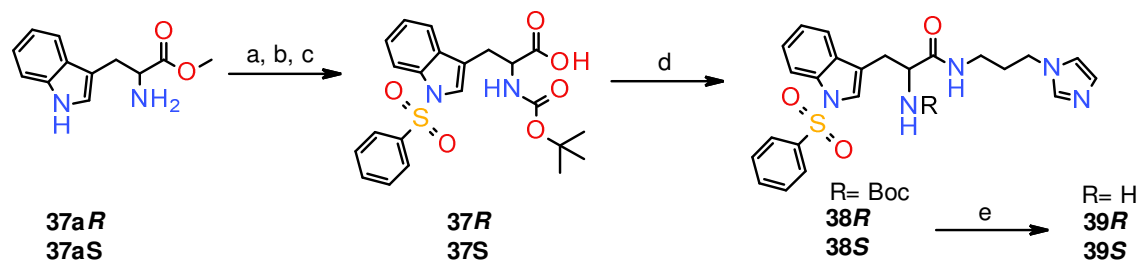
^a *Reactants and conditions:* a) NaOH, Bu_4NHSO_4 cat., DCM, Benzenesulfonyl chloride, room temp., 2 h; b) HCl 4N, dioxane, room temp., 16 h, 96% (2-step yield); c) 4-nitrophenyl chloroformate, Et_3N , 3-imidazol-1-yl-propylamine, THF, 4 h, room temp., 73%.



Scheme 3. Synthesis of the indole derivative **33**, **35** and **36**.^a

^a *Reactants and conditions:* a) imidazole, NaH, DMF, 80°C, 1h. b) NaOH, H₂O/EtOH, room temp., 30 min, 96% (2-step yield); c) H₂SO₄, MeOH, room temp., 2 h then NaOH, Bu₄NHSO₄ cat., DCM, benzenesulfonyl chloride, room temp., 3 h d) NH₄Cl, HOBt/EDCI, DIPEA, DMF, room temp., overnight, 71% (2-step yield); e) NaBH₄, AlCl₃, DME, room temp., 30 min, 45%; f) **33b**, HOBt/EDCI, DIPEA, DMF, room temp., overnight, 52%.

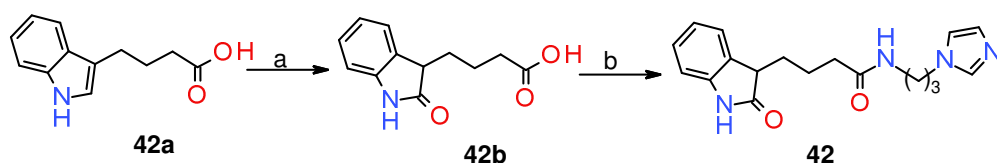
Branched analogues **38-39** were synthesized from tryptophan methyl esters **37a** as described in Scheme 4. The amine function was Boc-protected before sulfonylation of the indole. Then carboxylic acids **37** obtained after ester hydrolysis, reacted with 1-(3-aminopropyl)-imidazole as previously described. The Boc group was removed using HCl 4N in dioxane. Intermediates **37** protected analogues **38** and final amide **39** were all biologically tested.



Scheme 4. Synthesis of indole derivatives **37-39** from tryptophan.^a

^a *Reactants and conditions:* a) Boc₂O, Et₃N, DCM, room temp., 2 h b) NaOH, Bu₄NHSO₄ cat., DCM, benzenesulfonyl chloride, room temp., 3 h c) NaOH, MeOH/H₂O, room temp., overnight, 85-87% (3-step yield); d) 1-(3-aminopropyl)-imidazole, HOBt/EDCI, DIPEA, DMF, room temp., overnight, 74-83%; e) HCl 4N in dioxane, 0 °C overnight, 60-63%.

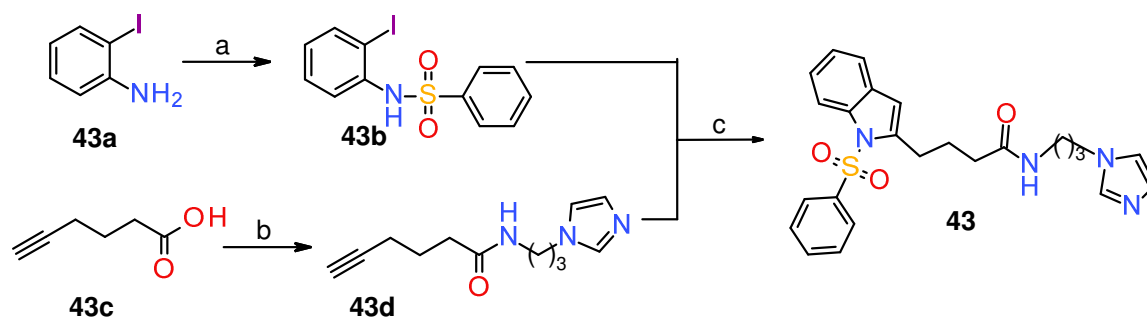
The 2-oxoindoline derivative **42** was obtained in two steps (Scheme 5). First, oxidation of the indolebutyric acid **42a** by conc. HCl in DMSO at room temperature for 30 min provided carboxylic acid **42b** that reacted with 1-(3-aminopropyl)-imidazole to give **42**.



Scheme 5. Synthesis of the compound **42**.^a

^a *Reactants and conditions:* a) DMSO, conc. HCl, room temp., 30 min., 78%; b) 1-(3-aminopropyl)-imidazole, HOBT, EDCI, DIPEA, DCM, room temp., 6 h, 14%.

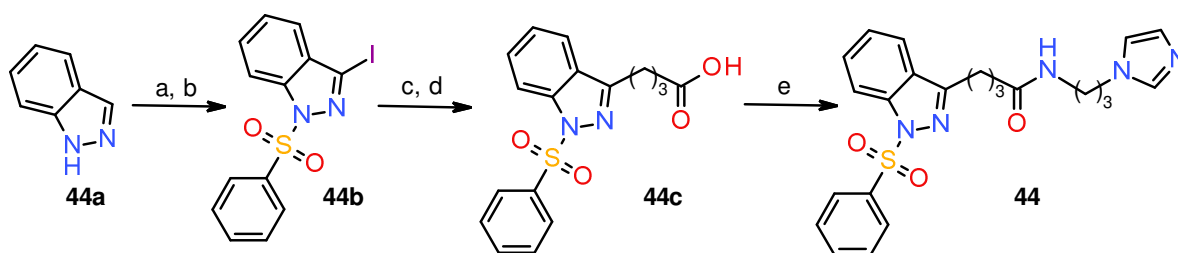
The 2-substituted indole regioisomer (**43**) was synthesized as described in Scheme 6. First 2-iodo-aniline **43a** was reacted with benzenesulfonylchloride in pyridine at room temperature overnight. Alkyne **43d** was obtained from carboxylic acid **43c** and 1-(3-aminopropyl)-imidazole using T3P®. Alkyne **43d** and iodide **43b** underwent Sonogashira coupling and subsequent intra-molecular cyclisation using CuI, Pd(PPh₃)₂Cl₂, trimethylamine in DMF under microwave irradiation at 80 °C for 20 min, to give analogue **43** (Scheme 6).



Scheme 6. Synthesis of the compound **43**.^a

^a *Reactants and conditions:* a) benzenesulfonyl chloride, pyridine, room temp., overnight, 90%; b) 1-(3-aminopropyl)-imidazole, T3P®, DIPEA, EtOAc, room temp., 4 h, 75%. c) CuI, Pd(PPh₃)₂Cl₂, Et₃N, DMF, microwave irradiation 80 °C, 20 min, 23%.

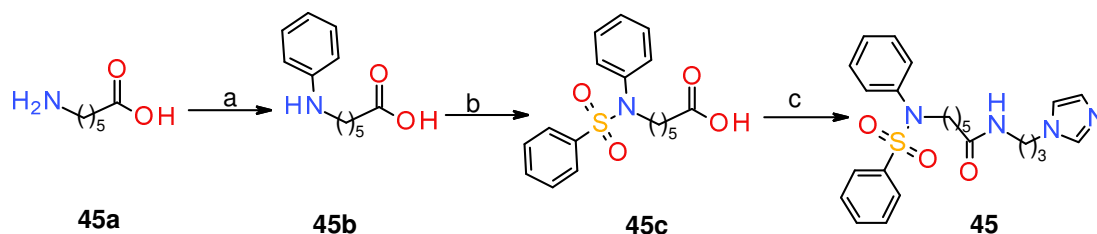
An indazole derivative **44** was synthesized by iodation of the indazole using iodine, KOH in DMF at room temperature followed by a nitrogen substitution by benzenesulfonylchloride to give **44b** (Scheme 7). Then, ethyl 4-bromobutyrate reacted with **44b** under a Ni-catalyzed Negishi coupling before saponification to afford **44c**. The latter was then converted to amide **44** by reaction with 1-(3-aminopropyl)-imidazole (Scheme 7).



Scheme 7. Synthesis of the compound **44**.^a

^a *Reactants and conditions:* a) I₂, KOH, DMF, room temp., overnight. b) KOH, Bu₄NHSO₄ cat., DCM, room temp., 3 h, 99%; c) Zn, I₂, DMA, ethyl 4-bromobutyrate, NiCl₂(PPh₃)₂, room temp., overnight. d) NaOH, MeOH, H₂O, room temp., overnight, 48% (2-steps); e) 1-(3-aminopropyl)-imidazole, HOBT/EDCI, DIPEA, DCM, room temp., overnight; 66%.

Uncyclized analogue **45** was formed in 3 steps from 6-aminohexanoic acid **45a** (Scheme 8). Copper-catalyzed Ullman reaction with iodobenzene using, K₃PO₄·H₂O, CuI in deanol/H₂O allowed the formation of the carboxylic acid **45b**. N-sulfonylation and amide formation afforded **45**. (Scheme 8)

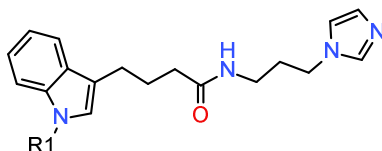


Scheme 8. Synthesis of the derivative **45**.^a

^a *Reactants and conditions:* a) Iodobenzene, K₃PO₄·H₂O, CuI, deanol, H₂O, 80 °C, overnight, 12%; b) Benzenesulfonyl chloride, pyridine, DCM, room temp., overnight, 90%; c) 1-(3-aminopropyl)-imidazole, HOBT, EDCI, DIPEA, DCM, room temp., overnight, 76%.

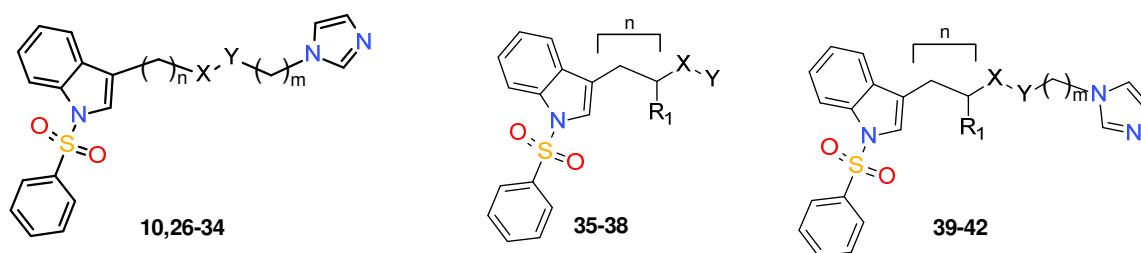
1.4. Structure-activity relationships.

Table 1 summarises the impact of the N-substitution of the indole. Removal (**2**) or replacement by an alkyl group (**3-4**), aryl (**5**) or benzyl (**6-7**) group of the sulfonylaryl group in **1** resulted in less potent or inactive compounds. Acylated analogue **8** retains some efficacy (2.40 activation fold at 100µM like **1**) but is less potent (pEC₅₀ = 4.67 vs 5.30 for **1**). The sulfonyl group was then maintained and diverse substituents were explored (**9-25**). Among N-sulfonylated indoles, replacement of the thiophene by a methyl (**9**) is tolerated. Fluorinated phenyl analogues (**11-13**) are more potent than phenyl analogue (**10**) but induce a lower activation fold. Substitution of the phenyl ring by chlorine (**14**), bromine (**15**), methyl (**16-17**) or methoxy (**18**) groups led to inactive and/or insoluble compounds. Regioisomer **19** is slightly less potent than hit **1**. Pyridyl analogue (**22**) is less potent than **1** but induces an activation fold of 3 at 100µM. Benzothiadiazolyl (**23**), biphenyl (**24**) and naphthyl (**25**) analogues are inactive. In comparison to **1**, analogue **10** was found to be more soluble (15 µM) while having a similar LogD (2.7 at pH 7.4).

Table 1: Impact of the *N*-substitution of the indole

Cpd #	R ₁	pEC ₅₀	Activation Factor @100 μM (30 μM)
1	2-thiophenyl-SO ₂ -	5.30±0.14	2.30±0.18
2	H-	4.05±0.40	1.35±0.51
3	Me-	— ^a	1.49
4	Et-	— ^a	1.21
5	Ph-	— ^a	(0.93) ^b
6	<i>p</i> -Chlorobenzyl-	— ^a	(0.76) ^b
7	<i>p</i> -Fluorobenzyl-	— ^a	(0.71) ^b
8	2-thiophenyl-CO-	4.67±0.27	2.40±0.05
9	Me- SO ₂ -	4.57±0.13	2.15±0.22
10	Ph- SO ₂ -	4.88±0.09	2.28±0.11
11	<i>o</i> -Fluorophenyl- SO ₂ -	5.10±0.16	(1.75±0.09) ^b
12	<i>m</i> -Fluorophenyl- SO ₂ -	4.99±0.06	(2.06±0.30) ^b
13	<i>p</i> -Fluorophenyl- SO ₂ -	5.17±0.27	(1.45±0.07) ^b
14	<i>p</i> -Chlorophenyl- SO ₂ -	— ^a	— ^a
15	<i>p</i> -Bromophenyl- SO ₂ -	— ^a	— ^a
16	<i>p</i> -Methylphenyl- SO ₂ -	— ^a	— ^a
17	<i>o</i> -Methylphenyl- SO ₂ -	— ^a	— ^a
18	<i>p</i> -Methoxyphenyl- SO ₂ -	— ^a	(1.08)
19	Thiophen-3-yl- SO ₂ -	4.86±0.14	1.97±0.25
20	1-Methylimidazol-4-yl- SO ₂ -	4.54±0.12	1.99±0.19
21	3,5-Dimethylisoxazol-4-yl- SO ₂ -	— ^a	1.50
22	3-Pyridyl- SO ₂ -	4.57±0.23	2.75±0.28
23	2,1,3-Benzothiadiazol- SO ₂ -4-yl-	— ^a	(1.50) ^b
24	<i>p</i> -Biphenyl- SO ₂ -	— ^a	(0.98) ^b
25	1-Naphthyl- SO ₂ -	— ^a	1.0

^aNot calculable; ^bNot soluble @100μM; n =4.

Table 2: Impact of the linker.

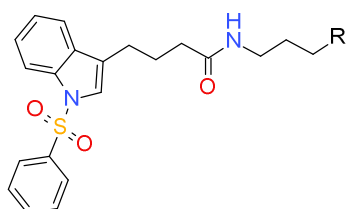
Cpd #	n	m	R ₁	X	Y	pEC ₅₀	Activation Factor @100 μM (30 μM)
10	3	3	-	CO	NH	4.88±0.09	2.28±0.11
26	0	3	-	CO	NH	_ ^a	(0.96) ^b
27	1	3	-	CO	NH	_ ^a	1.15
28	2	3	-	CO	NH	4.81± 0.15	1.64±0.05
29	4	3	-	CO	NH	5.14± 0.20	(1.71±0.07) ^b
30	3	4	-	CO	NH	5.09±0.21	2.67±0.11
31	3	2	-	CO	NH	_ ^a	1.0
32	3	3	-	CO	NMe	4.85±0.13	1.53±0.13
33	3	3	-	NH	CO	_ ^a	0.78
34	2	3	-	NHCO	NH	4.99±0.05	2.41±0.40
35	2	-	H	CO	NH ₂	_ ^a	0.94
36	2	-	H	CH ₂	NH ₂	_ ^a	1.1
37S	2	-	(S)NHBoc	CO	OH	_ ^a	1.23
37R	2	-	(R)NHBoc	CO	OH	_ ^a	1.29
38S	2	3	(S)NHBoc	CO	NH	_ ^a	_ ^c
38R	2	3	(R)NHBoc	CO	NH	_ ^a	_ ^c
39S	2	3	(S)NH ₂	CO	NH	4.84±0.11	2.28±0.12
39R	2	3	(R)NH ₂	CO	NH	_ ^a	1.54

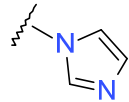
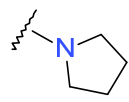
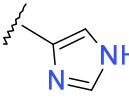
^aNot calculable; ^bNot soluble @100μM; ^cno dose-response effect; n=4.

In the phenylsulfonamide series, we explored the length, the branching and truncation of the linker and the amide function position (Table 2). Shortening of the linker by one, two or three methylene moieties (**26-28** and **31**) or elongation (**29-30**) seems to be tolerated for the efficacy but without improvement of the potency. The substitution of the amide with methyl group (**32**) reduce the potency of the compound. Replacement of the amide with a retro-amide (**33**) was detrimental for the activity. On the contrary, a urea linker (**34**) seems to be favourable for IDE activation activity but did not improve the potency (pEC₅₀) compared to the amide (**10**). In the absence of imidazolyl moiety (**35-37**), shortened basic (**35**), acid (**37**) or neutral (**36**) compounds did not allow to activate IDE. In the n=2 series and in the presence of the imidazolyl moiety (**28** and **38-39**), bulky substituent, like a NHBoc group, onto the

linker (**38**) decreased the activity while a primary amine substituent increased IDE activation, especially with the *S*-configuration (**39S**) compared to the *R*-configuration (**39R**) (Table 2).

Table 3: Impact of the replacement of the imidazole.

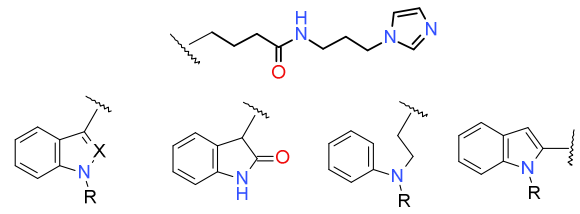


Cpd #	-R	pEC ₅₀	Activation Factor @100 μM (30 μM)	pKa ^a
10		4.88±0.09	2.28±0.11	6.9
40		— ^b	(0.78)	8.8
41		— ^b	1.27 ^a	7.0

^acalculated pKa using PipelinePilot™. ^bNot calculable; n=4.

In the initial screening, several analogues of **1**, displaying diverse alkyl groups, aromatic and heteroaromatic cycles, basic or not, in place of the N-(3-amino-propyl)imidazole moiety had been tested. Interestingly, only compound **1**, bearing a 1-imidazolyl ring, was found to be an IDE activator. Modification of the imidazole ring by other basic rings like 4-imidazolyl (**40**) or 1-pyrrolidyl (**41**) led to a loss of activity (Table 3).

Table 4: Impact regioisomerism or bioisosterism on the indole



Cpd #	pEC ₅₀	Activation Factor @100 μM (30 μM)
2	4.05±0.40	1.35±0.51
10	4.88±0.09	2.28±0.11
42	4.41±0.07	1.31±0.02
43	5.09±0.15	2.21±0.55
44	4.91±0.56	1.52±0.11
45	— ^a	— ^a

2: R=H; X=C
10: R=SO₂-Ph; X=C
44: R=SO₂-Ph; X=N
42: R=H; X=C
43: R=SO₂-Ph
45: R=SO₂-Ph

^a Not calculable. n=4.

Replacement of the indole core into oxindole (**42**), indazole (**44**) and aniline (**45**) rings led to a decreased activity while the two 2 and 3-substituted (**10**, **43**) indole regioisomers displayed equivalent activation fold (Table 4).

1.5. Docking of analogues **1**, **10**, **29-30**.

In order to model the binding sites of compound **1**, we first used the SiteFinder function of Molecular Operation Environment (MOE®, Chemical Computing Group, Montreal, Canada) within the crystallographic structure of the insulin-bound IDE (PDB code: 2WBY). We identified a putative binding pocket for **1** at the interface of the domains 2 and 4. This pocket happens to be the polyanion activation site of IDE [27],[40]. We thus further investigated the binding of **1** in this pocket using the 3D-structure of IDE bound to ATP (PDB Code 3TUV). In that structure, ATP forms potential hydrogen bonds with the side chains of Arg429 (domain 2), Lys898, Lys899 and Ser901 (domain 4), as shown in Supplementary Figure S6. The best redocked pose of ATP into the polyanion-binding site deviated 0.86 Å from the true crystal pose deposited in the PDB, denoting that the docking procedure was able to correctly predict binding of ATP and could be employed for further investigation of the binding of compound **1** and its analogues (Supplementary Fig. S7). **1** was docked into the activation site using the aforementioned procedure. As depicted in the Figure 7, the carbonyl function of the amide is engaged in a hydrogen bond with the terminal amine function of Lys899, while the nitrogen of the imidazole ring makes another hydrogen bond with the terminal amine of Lys898. Finally, the sulfonamide function interacts with Ser901, via its oxygens.

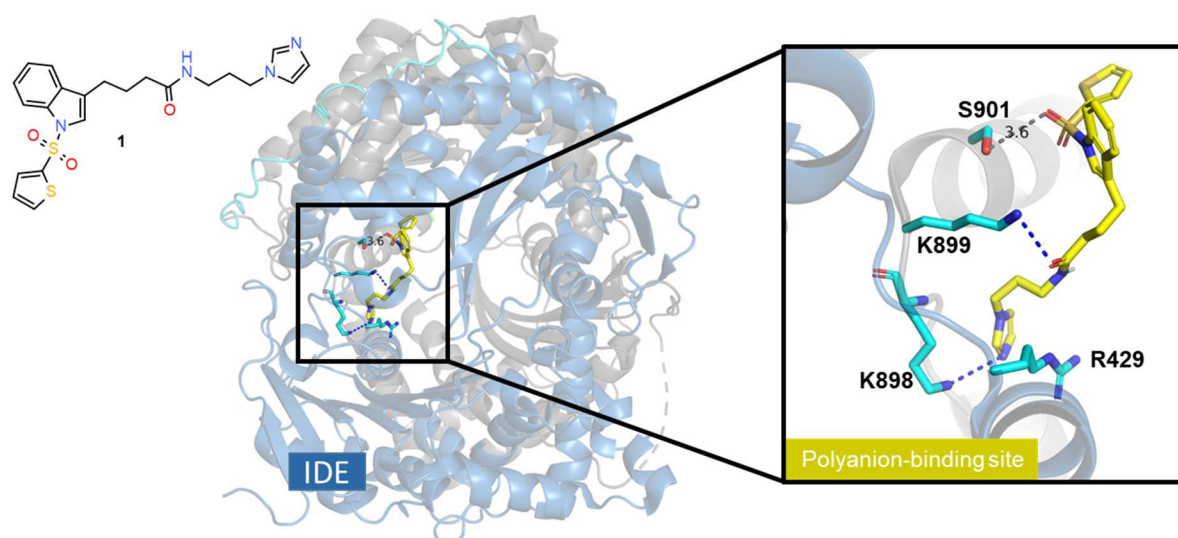


Figure 7: Interactions of **1** with IDE polyanion-binding site.^a

^a **1** is interacting with IDE via hydrogen bonds with the residues K898 and K899. (PDB code: 3TUV). IDE N-terminus and the C-terminus of IDE were colored blue and gray, respectively. Oxygens, nitrogens, sulfur are in red, blue and orange respectively. Carbons are colored in cyan for IDE and in yellow for compounds. Polar contacts and interactions are represented as blue dashed lines.

As the amide is playing a central role in the interaction of **1** with IDE, we decided to compare the binding of analogues **10**, **29** and **30** that differ from **1** by the presence of a phenyl instead of a thiophene on the indole part, and that display various amide linker lengths (Fig. 8). Compounds were docked using the same method in the polyanion-binding site of IDE. Interestingly, **10** binds slightly differently from **1** as the imidazole ring makes a hydrogen bond with the NH of the backbone of Lys898 instead of the side chain of the same residue (Fig. 8A). Elongation by one methylene moiety of the aliphatic linker between the indole and the amide (**29**, Fig. 8B) or between the amide and the imidazole (**30**, Fig. 8C), restores the hydrogen bond between imidazole and the nitrogen of the side chain of Lys898. The extension of the linker on the indole side in **29**, induces a new conformation that allows the carbonyl function of the amide to interact with side chain of Arg429 while Ser901 and Lys899 both make hydrogen bonds with the oxygens of the sulfonyl function (Fig. 8B). Interestingly, the analogue **30** is not able to make interactions with IDE via its amide function nor with Lys899 (Fig. 8C).

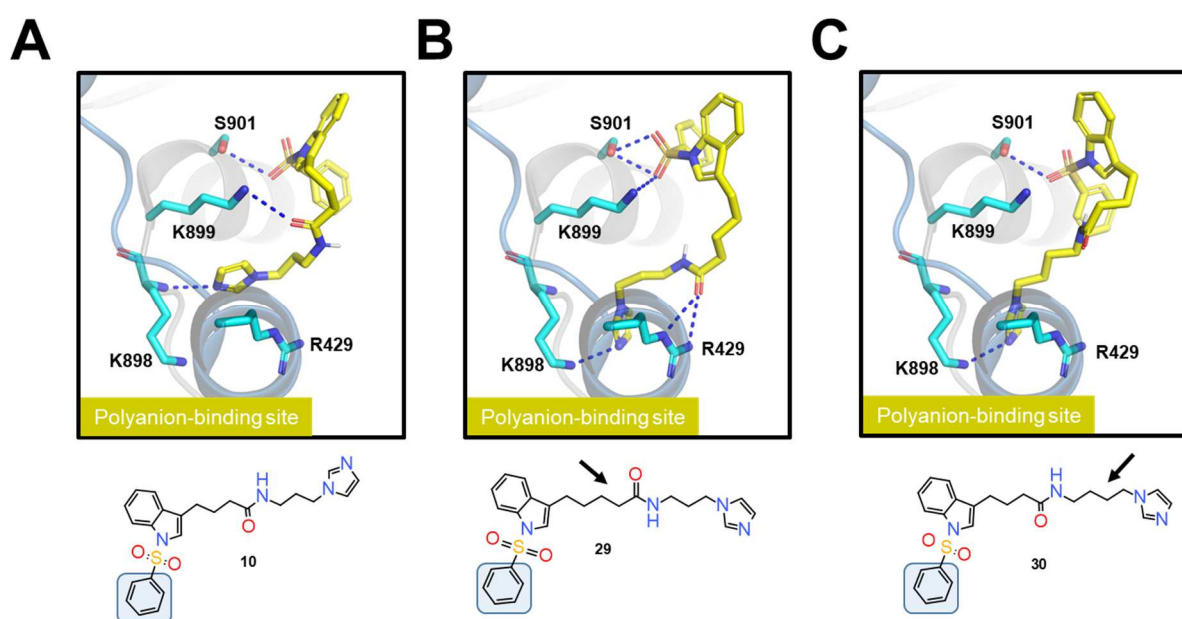


Figure 8: Docking poses of the 3 analogues **10** (A), **29** (B) and **30** (C) in the polyanion binding site of IDE (PDB code: 3TUV).^a

^a IDE N-terminus and the C-terminus of IDE were colored blue and gray, respectively. Oxygens, nitrogens, sulfur are in red, blue and orange respectively. Carbons are colored in cyan for IDE and in yellow for compounds. Polar

contacts and interactions are represented as blue dashed lines. These 3 compounds display a phenyl ring (in blue box) on the indole in place of a thiophene in **1**. Black arrows point the differences of chain length in **29** and **30** respectively to **10**.

1.6. Discussion.

IDE was first described for its high affinity and hydrolytic properties towards insulin. Initially, the development of inhibitors to lift glucose intolerance by increasing insulin half-life was thought to be a therapeutic option. However, inhibitors tested both *in vitro* and *in vivo* displayed contradictory effects on this phenotype and the characterization of tissue-selective IDE-ko animals underlined the complex role of IDE in glucose homeostasis [14],[13]. In particular some new non-proteolytic functions of IDE have been discovered [41]. In that context, and along with the need of activators of IDE regarding A β , activators of IDE regarding insulin could also be of interest.

Thanks to screening we identified an original chemical series of IDE activators of both A β and insulin hydrolysis. Here, activator **1** decreases glucose-stimulating insulin secretion in β -pancreatic cells as well as decreases insulin content. The latter can directly be linked to the catalytic activation of IDE by **1**.

The decrease of GSIS in β -pancreatic cells treated with **1** could be linked to the extracellular activation of IDE. Still, it is surprising that activator **1** decreases GSIS like IDE inhibitors. As both activators and inhibitors are IDE ligands, they may act on this phenotype by modulating non-proteolytic functions of IDE.

Structure-activity relationships for this indole activators series highlighted the importance of the sulfonylation of the nitrogen of the indole (Table 1). Thiophene, fluoro-phenyl and phenyl analogues are the most potent (Table 1) while thiophene, phenyl and pyridine derivatives are the most efficacious regarding the fold of hydrolysis (up to 300%). Elongation of the linker between indole and imidazole is better tolerated than shortening (Table 2). Introduction of an amine function in the side chain is tolerated in shorter chains (Table 2) and specific configuration. Both a moderate pKa (**10** > **40**) and a right positioning of nitrogens (**10** > **41**) are essential for activity (Table 3). Other heterocycles than indole are also tolerated (Table 4).

IDE-C half is predominantly positively charged while IDE-N half is predominantly negatively charged. ATP, a substrate-dependent IDE activator, is thought to modify the IDE oligomer state or the open/close equilibrium of IDE by breaking the electrostatic forces between the two halves of IDE and

consequently increasing the population of open form facilitating the uptake of peptide substrates [42]. X-ray structure of IDE co-crystallized with ATP revealed that the adenine ring of ATP was disordered. Interactions of ATP with IDE are mediated through its triphosphate moiety. Lys898, Lys899 and Ser901 in domain 4 and Arg429 in domain2 make hydrogen bonds with this moiety via their side chain. Lys898 also interacts via its backbone to phosphate groups [27].

Docking is highly consistent with the structure-activity relationships as it shows that sulfonamide and imidazole are key for binding. All docked compounds are in the same activation range and it is not clear however how slightly different binding in the series affect potency or efficacy at this stage. Our docking study supports the idea that compounds in this series act through interaction with the polyanion-binding site. All compounds docked were shown to bind at least 3 of the 4 residues identified above. This supports the possibility to target this site by compounds displaying HBA in place of ionisable groups like phosphates. Previously **1a1** and **1a2** have been shown to interact with a site partially overlapping with the ATP binding site [36]. Here we provide a new chemical series with extensive binding in the polyanion-binding site. Interestingly also, and as opposed to other chemical series of activators, this series acts as an activator of both a short A β analogue (10-mer) and full-length insulin hydrolysis.

2. Conclusion

Thanks to screening, we identified drug-like activators of IDE. Hit **1** (**BDM35899**) was shown to be an activator of both short A β -like peptide and full-length insulin hydrolysis by IDE. **1** was characterized in cells regarding glucose-stimulating insulin secretion. Structure-Activity Relationships highlighted the key role of the sulfonamide function and the imidazole ring in the activation of IDE. This role was confirmed by docking studies that showed an extended binding in the polyanion binding site of IDE, in an original, unprecedented mode. This series constitute an interesting starting point for future optimization of activators of IDE as tools to explore the multiple roles of IDE.

3. Experimental section

3.1. *In vitro* IDE activity assays

Recombinant IDE was a gift from Pr W.J. Tang, BenMay institute, University of Chicago, USA [24]. Ac-Cys-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Trp-NH₂ was synthesized by NeoMPS. *In vitro* IDE activity was

measured with a quenched substrate ATTO 655-Cys-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Trp (Km 100 μ M) or with insulin (Actrapid®).

3.1.1. Screening assay with quenched substrate

Human IDE (0.05 μ g/incubate) was incubated 10 min at room temperature with compound (final concentration 30 μ M, 1% (v/v) DMSO final) in Hepes sodium 50 mM, NaCl 100 mM, pH 7.4 and the enzymatic reaction is started by adding the quenched substrate (final concentration 5 μ M). After 20 min at 37 °C, samples (1% (v/v) DMSO final) are excited at 635 nm and fluorescence emission at 750 nm is measured on a Victor3 V1420 Perkin Elmer spectrophotometer during 2 h every 2 minutes. Initial velocities were calculated on the linear phase of the enzymatic kinetic curve (usually 8 to 10 time points). Suramin was used as reference at 10 μ M. 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.

3.1.2. Dose-Response Curves with quenched substrate

Percentage of activation at each concentration were obtained as for screening. All measurements were carried out as 8-point dose response curves (30 nM to 100 μ M usually with constant 1% DMSO (v/v)) and reported as the average of at 4 independent repeats of duplicates.. Data analysis was performed using Xlfit® v 5.0 and GraphPad Prism® v 4.0. Nonlinear curve fitting and statistical analysis was done using built-in functions.

3.1.3. Hydrolysis of unlabeled insulin by IDE

Insulin (Actrapid®) was dissolved in Hepes 50 mM with 100 mM NaCl, pH 7.4. Then, 20 μ L of IDE at 30 μ g/mL were pre-incubated 1 or 10 minutes at 37 °C with 20 μ L of vehicle, compound or inhibitor (EDTA) in microtiter plates (black, low-binding). The reaction was started with the addition of 40 μ L of substrate at 4 μ M. Final concentrations of IDE and insulin, were 7.51 μ g/mL and 1 μ M respectively. The reaction was quenched at a suitable time point with 20 μ L of a solution of TFA/ACN 0.1%. Quantification of residual substrate was performed by HPLC analysis (UV 215 nm) using an UltiMate™ nano LC system apparatus, equipped with a Famos® autosampler and a Switchos™ precolumn switching device all from LC_packings (Dionex.Corp). 10 μ L of sample were loaded on a precolumn 5*0.3 mm equipped with a guard cartridge Q95961 10*1.0 mm from Interchim at a flowrate of 50 μ L/min for preconcentration. Separation was performed using an Uptisphere UP5WTF-A10 C18wtf 5- μ m particle size column,

dimensions 0.3 * 100 mm from Interchim at 50 °C. A gradient starting from 75% H₂O/0.1% TFA (v/v) and reaching 75% CH₃CN /0.09% TFA (v/v) within 10 min at a flow rate of 0.3 µL/min was used and quantification was done at 215/254 nm.

3.2. Cellular assays

3.2.1. Cell culture

Min 6 cells (provided by professor Jurgen Puentner (Sanofi Aventis, Germany)) were cultured in a humidified atmosphere containing 5% CO₂ in DMEM glutamax medium (Gibco) (containing 25mmol/L glucose) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Corning), β-mercapto-Ethanol 50 µM (Sigma), and gentamycin 1% (Invitrogen).

3.2.2. Glucose Stimulated Insulin Secretion (GSIS):

Min6 were seeded in 24 well plate (300 000 cells/well) and cultured for 7 days. The cells are pre-treated for 24h before or 1h during GSIS. Each compound is dissolved in DMSO. Before the GSIS experiment, the cells were washed and preincubated for 1 hour at 37 °C in glucose-free Krebs-Ringer bicarbonate HEPES buffer (KRBH) of the following composition: 115 mM NaCl, 4.7 mM KCl, 2.6 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 20 mM NaHCO₃, 16mM HEPES adjusted pH 7.4, BSA free fatty acid 0.5% (w/v). Next the cells are stimulated in KRBH buffer containing 2.8 mM of glucose or 20 mM of glucose for 1 hour. Incubation was stopped by putting the plate on the ice, the supernatant was collected for insulin secretion. Insulin secretion was measured by ELISA (Mercodia).

3.3. *in vitro* ADME

3.3.1. General information.

Chromatography was performed using a UPLC system, an Acquity I-Class (Waters). The separation was carried out on a Waters Acquity BEH C18 column (2.1 mm × 50 mm, 1.7 µm). The temperatures of the autosampler and column oven were 10°C and 40°C respectively and the sample injection volume was 1 µL. The mobile phase consisted of 0.1% formic acid in water as solvent A and 0.1% formic acid in acetonitrile as solvent B at a flow rate of 600 µL/min. The gradient was as follows: 0 - 0.2 min (98% A and 2% B), 2 - 2.5 min (2% A and 98% B), 2.6 min (98% A and 2% B), 4 min (98% A and 2% B). The gradient step was linear.

Mass spectrometry was performed using a Xevo TQD mass spectrometer (Waters). Detection of the analytes was performed by electrospray ionization (ESI) in positive mode with the appropriate MRM transition. Capillary tension, cone tension and collision energy were optimized for each compound;

the desolvation temperature was set at 600 °C, and the desolvation and cone gas flow rate respectively set at 1200 L / h and 50 L / h. The LC-MS/MS instrument was controlled by MassLynx software (Waters).

3.3.2. Solubility/LogD measurements.

Briefly, 10 µL of a 10 mM solution in DMSO of the compound are diluted either in 490µL of PBS pH 7.4 or in organic solvent MeOH in a 700 µL-microtube (in triplicate). The tubes are gently shaken 24h at room temperature, then centrifuged for 5 minutes at 4000 rpm. The mixtures are filtered over 0.45 µm filters (Millex-LH Millipore). 20 µL of sample are diluted in 180 µL of MeOH. The solubility is determined by the ratio of mass signal area PBS/ organic solvent. 40µL of a 10 mM solution in DMSO of the compound were diluted in 1.960 mL of a 1/1 octanol /PBS at pH 7.4 mixture. The mixture was gently shaken 2 h at room temperature. 20 µL of each phase was diluted in 480 µL of MeOH and analyzed by LC-MS. Each compound is tested in triplicate. Log D was determined as the logarithm of the ratio of concentration of product in octanol and PBS respectively, determined by mass signals.

3.3.3. Stability in plasma

Incubations were performed in duplicate in Eppendorf tubes. The plasma (mouse plasma, lithium heparinated, from Sera Laboratories International Ltd) was pre-incubated 5 min at 37 °C before the addition of test compounds to a final concentration of 10µM (1% DMSO maximum). At the defined time points, 50 µL from each tube were removed to another tube containing 450 µL of cold CH₃CN + internal standard (1µM). After centrifugation (10 min at 10000 rpm), supernatants are analyzed. The degradation half-life (t_{1/2}) values were calculated using the following equation: $t_{1/2} = 0.693/k$ where k is the first-order degradation rate constant. The degradation rate constant (k) was estimated by one-phase exponential decay non-linear regression analysis of the degradation time course data using Xlfit software (version 2.1.2 or higher) from IDBS.Ltd.

3.4. Docking

A 3D structure of the ATP-bound IDE was downloaded from Protein Data Bank (PDB entry: 3TUV, rcsb.org) [43]. All implicit hydrogens (of the protein and the ligand) were added using MOE® 2020.09 (Chemical Computing Group ULC, 1010 Sherbrooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7). Water molecules that take part in at least three hydrogen bonds with the protein and/or the ligand, at least 2 of which are with the protein, were kept. Other water molecules were deleted. The protein structure was saved as a moe file. Hydrogen bonds were automatically detected by the programs MOE

2020.09 and PyMOL® (Delano, W. L. The PyMOL Molecular Graphics System. DeLano Scientific LLC: San Carlos, CA, 2002). The 2D structures of ligands were constructed with Biovia Draw (Dassault Systèmes, Vélizy-Villacoublay, France), and their energy minimization was carried out with the MMFF94 function implemented in MOE. All output ligands were saved in the mdb file format. A rigid molecular docking procedure was carried out using MOE 2020.09. The binding site was defined with “Ligand Atoms” mode in which the active ligand was ATP. A pharmacophore model consisting of 3 features denoted by the two aromatic rings and the oxygen bound to the two first phosphorus atoms of the ATP was applied (Supplementary Figure S7). The AMBER10 forcefield and the method Pharmacophore were used. A maximal number of 5 poses were retained for each ligand. Post-docking interactions of all ligands with the protein were analyzed with MOE 2020.09 and were rendered in PyMOL®.

3.5. Chemical synthesis

3.5.1. 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. 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 ^{13}C 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 μm column C₁₈ Dynamax (250 mm \times 41.4 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.

3.5.2. General procedure A.

To a solution of the specific commercial indole acid derivative (**49a-49d**) (1.0 eq.) in MeOH was added H₂SO₄ dropwise (3.3 eq.) with controlled temperature below 45 °C. The mixture was stirred at room temperature for 2h, then poured onto H₂O, adjusted to pH ~ 8 with NaHCO₃ and extracted twice with EtOAc. The combined organic layers were washed with H₂O and brine, dried over MgSO₄, filtered and concentrated to give the desired products (**50a-50d**). The crude product (**50a-50d**) and the aldehyde indole derivative (**49e**) were then solubilized in DCM of DMF and NaOH (3.0 eq.) and Bu₄NHSO₄ (0.05 eq.) were added and left to stir 10 min at room temperature before the halide or sulfonyl chloride derivative or the halogenated acyl (1.05 eq.) was added and left to stir overnight at room temperature. The solid was filtered off. The filtrate was concentrated and dissolved in MeOH/THF (2/1 (v/v)). Aqueous solution of NaOH (3 mL) was added. The mixture was stirred at room temperature for 3h. It was diluted with H₂O and adjusted to pH ~ 4 with 1M KSO₄ solution. The precipitate was collected with filter, washed with H₂O, and dried in a vacuum desiccator to afford the desired products (**51a-51d**). To the mixture of (**51a-51e**), HOBt (1.5 eq.) and EDCI (2.5 eq.) in DMF was added DIPEA (4.0 eq.). After stirred for 10 min at room temperature, the amine (1.5 eq.) was added and the mixture was left to stir overnight at room temperature. DMF was removed under reduced pressure and the residue was diluted with DCM, successively washed twice with aqueous saturated NaHCO₃ solution, brine, dried over MgSO₄, filtered

and concentrated under reduced pressure. The residue was to afford the desired product (**1-32** and **43-44**).

3.5.3. General procedure B for the synthesis of analogues **41-42**.

Tryptophan methyl ester hydrochloride (**37a-37b**) were suspended in DCM. To the suspension was added TEA (4.0 eq.), followed by di-tert-butyl dicarbonate (1.2 eq.) and left to stir at room temperature for 2h. It was diluted with DCM, washed twice with saturated NaHCO₃ and brine, dried over MgSO₄, filtered and concentrated to afford the desired product as white solid. LC-MS: tr = 2.97 min, m/z 317.0 [M-H]⁻, 319.0 [M+H]⁺. A mixture of *N*-Boc-tryptophan methyl ester, NaOH (3.0 eq.) and Bu₄NHSO₄ (0.05 eq.) in DCM was stirred for 5 min at room temperature before benzenesulfonyl chloride (1.05 eq.) was added and left to stir 2h at room temperature. DCM was removed under reduced pressure and to the residue were added NaOH (1.15 eq.), MeOH, H₂O and THF. The mixture was stirred overnight at room temperature. It was adjusted to pH ~5 with acetic acid. Most of the organic solvents were removed under reduced pressure. The remained aqueous residue was extracted with DCM. The combined organic layer was washed with H₂O and brine, dried over MgSO₄, filtered and concentrated to afford the desired product (**37-38**) as off-white solid. To the mixture of (**37-38**), HOBT (1.5 eq.) and EDCI (2.0 eq.) in DMF was added DIPEA (4.0 eq.). After stirred for 10 min at room temperature, 1-(3-aminopropyl)-imidazole (1.5 eq.) was added. The mixture was stirred at room temperature over the week-end. DMF was removed under reduced pressure. The residue was diluted with DCM, successively washed twice with saturated NaHCO₃ and brine, dried over MgSO₄, filtered and concentrated by under reduced pressure. It was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired products (**39-40**) as white powders. The Boc-protected compound (**39-40**) was dissolved into HCl 4M in dioxane at 0°C and left to stir at room temperature overnight. Dioxane was removed under reduced pressure before the residue was dissolved in H₂O, basified with NH₄OH and extracted with DCM. The organic layer washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The residues were purified by preparative LC-MS with pH 9 buffer solution as the eluent to give the desired products (**41-42**) as an oil.

3.5.4. Compounds **1-48**.

3.5.4.1. 4-[1-(thiophene-2-sulfonyl)-1*H*-indol-3-yl]-*N*-(3-imidazol-1-yl-propyl)-butyramide (**1**).

1 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product

as pale-yellow sticky oil (0.78 mmol, 29%). LC-MS: tr = 2.47 min, 100% purity (at 215 nm), m/z 454.9 [M-H]⁻, 500.9 [M-H+2Na]⁻, 456.9 [M+H]⁺. HRMS m/z calculated for C₂₂H₂₅N₄O₃S₂ [M + H]⁺ 457.1368, found 457,1366. ¹H NMR (300 MHz, CD₃OD): δ 1.96 (4H, m), 2.25 (2H, t, J = 7.5 Hz), 2.73 (2H, t, J = 7.5 Hz), 3.16 (2H, t, J = 6.9 Hz), 4.04 (2H, t, J = 6.9 Hz), 6.97 (1H, s), 7.05 (1H, dd, J = 3.9, 5.1 Hz), 7.14 (1H, s), 7.27 (1H, t), 7.35 (1H, t), 7.39 (1H, s), 7.55 (1H, d, J = 7.5 Hz), 7.66 (1H, s), 7.73 (1H, m), 7.95 (1H, d, J = 8.1 Hz). ¹³C NMR (75 MHz, CD₃OD): δ 25.2, 26.3, 31.9, 36.4, 37.5, 45.6, 115.0, 120.6, 120.7, 124.1, 124.7, 125.0, 125.9, 128.6, 129.1, 132.5, 134.5, 135.3, 136.8, 138.5, 139.0, 175.8.

3.5.4.2. *N*-(3-imidazol-1-ylpropyl)-4-(1*H*-indol-3-yl)butanamide (**2**).

2 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as pale-yellow sticky oil (0.38 mmol, 37%). LC-MS: tr = 3.97 min, >99% purity (at 215 nm), m/z 311.1 [M+H]⁺. HRMS m/z calculated for C₁₈H₂₃N₄O [M + H]⁺ 311.1872, found 311,1874. ¹H NMR (300 MHz, DMSO-*d*₆): δ: 1.76-1.92 (m, 4H), 2.14 (t, J = 7.5 Hz, 2H), 2.67 (t, J = 7.5 Hz, 2H), 3.00 (dd, J = 6.8 and 5.8 Hz, 2H), 3.95 (t, J = 6.8 Hz, 2H), 5.89 (s, 1H), 6.92-6.97 (m, 1H), 7.02-7.07 (m, 1H), 7.08-7.11 (m, 1H), 7.18 (s, 1H), 7.30-7.34 (m, 1H), 7.49 (br d, J = 7.7 Hz, 1H), 7.65 (s, 1H), 7.86 (t, J = 5.6 Hz, 1H), 10.77 (s, 1H); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 24.4, 26.3, 30.8, 35.4, 35.7, 44.0, 111.5, 114.2, 118.2, 118.4, 119.7, 121.0, 122.4, 127.3, 127.7, 136.4, 137.3, 172.5

3.5.4.3. 4-(1-methyl-1*H*-indol-3-yl)-*N*-(3-imidazol-1-yl-propyl)-butyramide (**3**).

3 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as pale-yellow sticky oil 0.66 mmol, 33%). LC-MS: tr = 2.08 min, 99% purity (at 215 nm), m/z 323.2 [M-H]⁻, 369.1 [M-H+2Na]⁻, 325.0 [M+H]⁺. HRMS m/z calculated for C₁₉H₂₅N₄O [M + H]⁺ 325.2028, found 325,2020. ¹H NMR (300 MHz, CD₃OD): δ 1.86 (2H, quint, J = 6.9 Hz), 1.98 (2H, quint, J = 7.5 Hz), 2.23 (2H, t, J = 7.5 Hz), 2.74 (2H, t, J = 7.5 Hz), 3.65 (3H, s), 3.91 (2H, t, J = 6.9 Hz), 6.88 (1H, s), 6.94 (1H, s), 7.01 (1H, t, J = 7.5 Hz), 7.04 (1H, s), 7.13 (1H, t, J = 7.5 Hz), 7.23 (1H, d, J = 8.1 Hz), 7.53 (1H, d, J = 7.8 Hz), 7.58 (1H, s). ¹³C NMR (75 MHz, CD₃OD): δ 24.3, 26.4, 30.5, 35.5, 36.0, 40.1, 44.1, 108.9, 113.9, 118.1, 118.5, 119.2, 120.9, 124.5, 127.7, 128.1, 136.2, 137.1, 174.9.

3.5.4.4. 4-(1-ethyl-1*H*-indol-3-yl)-*N*-(3-imidazol-1-yl-propyl)-butyramide (**4**).

4 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired

product as pale-yellow sticky oil (1.53 mmol, 66%). LC-MS: tr = 2.17 min, 99% purity (at 215 nm), *m/z* 337.3 [M-H]⁻, 383.1 [M-H+2Na]⁻, 339.1 [M+H]⁺. HRMS *m/z* calculated for C₂₀H₂₇N₄O [M + H]⁺ 339.2185, found 339,2182. ¹H NMR (300 MHz, CD₃OD): δ 1.32 (3H, t, *J* = 7.2 Hz), 1.86 (2H, quint, *J* = 6.9 Hz), 1.99 (2H, quint, *J* = 7.5 Hz), 2.24 (2H, t, *J* = 7.5 Hz), 2.75 (2H, t, *J* = 7.5 Hz), 2.75 (2H, t, *J* = 7.5 Hz), 3.10 (2H, t, *J* = 6.9 Hz), 3.90 (2H, t, *J* = 6.9 Hz), 4.06 (2H, t, *J* = 7.2 Hz), 6.94 (1H, s), 6.95 (1H, s), 7.00 (1H, t, *J* = 7.2 Hz), 7.03 (1H, s), 7.13 (1H, t, *J* = 7.2 Hz), 7.26 (1H, d, *J* = 8.1 Hz), 7.51 (1H, d, *J* = 7.8 Hz), 7.57 (1H, s). ¹³C NMR (75 MHz, CD₃OD): δ 14.6, 24.3, 26.5, 30.5, 35.5, 36.0, 40.1, 44.1, 108.9, 113.9, 118.2, 118.5, 119.2, 120.9, 124.5, 127.7, 128.1, 136.2, 137.1, 174.9.

3.5.4.5. 4-(1-benzyl-1*H*-indol-3-yl)-*N*-(3-imidazol-1-yl-propyl)-butyramide (**5**).

5 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as pale-yellow sticky oil (1.22 mmol, 60%). LC-MS: tr = 2.43 min, 99% purity (at 215 nm), *m/z* 399.0 [M-H]⁻, 445.0 [M-H+2Na]⁻, 401.0 [M+H]⁺. HRMS *m/z* calculated for C₂₅H₂₉N₄O [M + H]⁺ 401.2341, found 401,2339. ¹H NMR (300 MHz, CD₃OD): δ 1.84 (2H, quint, *J* = 6.9 Hz), 1.99 (2H, quint, *J* = 7.5 Hz), 2.24 (2H, t, *J* = 7.5 Hz), 2.75 (2H, t, *J* = 7.5 Hz), 3.08 (2H, t, *J* = 6.9 Hz), 3.87 (2H, t, *J* = 6.9 Hz), 5.14 (2H, s), 6.94 (1H, s), 6.95 (1H, s), 6.97-7.08 (5H, m), 7.11-7.20 (4H, m), 7.53 (1H, d, *J* = 6.9 Hz), 7.56 (1H, s). ¹³C NMR (75 MHz, CD₃OD): δ 24.3, 26.4, 30.5, 35.5, 36.0, 44.1, 49.2, 109.5, 114.5, 118.5, 118.6, 119.2, 121.3, 125.7, 126.6, 127.0, 127.7, 128.2, 128.3, 136.8, 137.1, 138.3, 174.9.

3.5.4.6. 4-[1-(4-chlorobenzyl)-1*H*-indol-3-yl]-*N*-(3-imidazol-1-yl-propyl)-butyramide (**6**).

6 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 22/1) to afford the desired product as light-yellow sticky oil (0.57 mmol, 48%). LC-MS: tr = 2.72 min, 97% purity (at 215 nm), *m/z* 433.0 [M-H]⁻, 479.0 and 481.0 [M-H+2Na]⁻, 434.9 and 436.9 [M+H]⁺. HRMS *m/z* calculated for C₂₅H₂₈ClN₄O [M + H]⁺ 435.1952, found 435,1952. ¹H NMR (300 MHz, CD₃OD): δ 1.87 (2H, quint, *J* = 6.9 Hz), 2.00 (2H, quint, *J* = 7.5 Hz), 2.25 (2H, t, *J* = 7.2 Hz), 2.76 (2H, t, *J* = 7.5 Hz), 3.10 (2H, t, *J* = 6.9 Hz), 3.92 (2H, t, *J* = 6.9 Hz), 5.14 (2H, s), 6.94-6.99 (4H, m), 7.00-7.09 (3H, m), 7.12-7.18 (3H, m), 7.56 (1H, d, *J* = 7.5 Hz), 7.58 (1H, s). ¹³C NMR (75 MHz, CD₃OD): δ 24.3, 26.3, 30.5, 35.5, 36.0, 44.2, 109.4, 114.8, 118.6, 119.2, 121.4, 125.6, 127.7, 128.1, 128.26, 128.29, 132.7, 136.6, 137.1, 174.9.

3.5.4.7. 4-[1-(4-fluorobenzyl)-1*H*-indol-3-yl]-*N*-(3-imidazol-1-yl-propyl)-butyramide (**7**).

7 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as light-yellow sticky oil (0.56 mmol, 54%). LC-MS: tr = 2.60 min, 100% purity (at 215 nm), *m/z* 417.0 [M-H]⁻, 463.0 [M-H+2Na]⁻, 419.0 [M+H]⁺. HRMS *m/z* calculated for C₂₅H₂₈N₄O [M + H]⁺ 419.2247, found 419,2244. ¹H NMR (300 MHz, CD₃OD): δ 1.90 (2H, quint, *J* = 6.9 Hz), 2.00 (2H, quint, *J* = 7.5 Hz), 2.26 (2H, t, *J* = 7.5 Hz), 2.77 (2H, t, *J* = 7.5 Hz), 3.12 (2H, t, *J* = 6.9 Hz), 3.96 (2H, t, *J* = 6.9 Hz), 5.22 (2H, s), 6.91-7.04 (5H, m), 7.06-7.11 (4H, m), 7.21 (1H, d, *J* = 8.1 Hz), 7.56 (1H, d, *J* = 7.5 Hz), 7.61 (1H, s). ¹³C NMR (75 MHz, CD₃OD): δ 24.2, 26.3, 30.5, 35.5, 36.0, 44.2, 109.4, 114.6, 114.7, 115.0, 118.5, 119.2, 121.3, 125.5, 127.7, 128.2, 128.3, 128.4, 134.3, 134.4, 136.7, 160.5, 163.7, 174.9.

3.5.4.8. *N*-(3-imidazol-1-ylpropyl)-4-[1-(thiophene-2-carbonyl)indol-3-yl]butanamide (**8**).

8 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 15/1) to afford the desired product as pale yellow sticky oil (0.32 mmol, 46%). LC-MS (10 min): tr = 4.78 min, purity >95% (at 215 nm), *m/z* 421.0 [M+H]⁺. HRMS *m/z* calculated for C₂₃H₂₅N₄O₂S [M + H]⁺ 421.1698, found 421,1697. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.81-1.97 (m, 4H), 2.16 (t, *J* = 7.5 Hz, 2H), 2.70 (t, *J* = 7.5 Hz, 2H), 3.05 (dd, *J* = 12.7 and 6.9 Hz, 2H), 4.41 (t, *J* = 6.9 Hz, 2H), 7.21-7.25 (m, 2H), 7.29-7.34 (m, 1H), 7.64-7.67 (m, 3H), 7.9 (dd, *J* = 3.8 and 1.0 Hz, 2H), 8.02 (dd, *J* = 4.9 and 1.4 Hz, 1H), 8.07 (dd, *J* = 4.9 and 1.0 Hz, 1H), 8.23-8.27 (m, 2H), 8.02 (dd, *J* = 3.8 and 1.2 Hz, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 23.7, 24.7, 30.8, 34.9, 35.6, 46.0, 115.8, 119.3, 121.7, 123.6, 124.3, 124.8, 127.7, 128.0, 128.3, 129.3, 130.6, 134.0, 135.9, 136.5, 124.2, 161.0, 171.9.

3.5.4.9. 4-(1-methylsulfonyl)-1*H*-indol-3-yl)-*N*-(3-imidazol-1-yl-propyl)-butyramide (**9**).

9 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 15/1) to afford the desired product as pale-yellow sticky oil (0.40 mmol, 42%). LC-MS: tr = 2.05 min, 100% purity (at 215 nm), *m/z* 387.0 [M-H]⁻, 433.0 [M-H+2Na]⁻, 388.9 [M+H]⁺. HRMS *m/z* calculated for C₁₉H₂₅N₄O₃S [M + H]⁺ 389.1647, found 389,1647. ¹H NMR (300 MHz, CD₃OD): δ 1.96 (4H, m), 2.28 (2H, t, *J* = 7.5 Hz), 3.11 (3H, s), 3.17 (2H, t, *J* = 6.9 Hz), 4.01 (2H, t, *J* = 6.9 Hz), 6.95 (1H, s), 7.11 (1H, s), 7.27 (1H, t, *J* = 7.5 Hz), 7.29 (1H, s), 7.34 (1H, t, *J* = 7.5 Hz), 7.60 (1H, d, *J* = 7.5 Hz), 7.64 (1H, s), 7.85 (1H, d, *J* = 7.5 Hz). ¹³C NMR (75 MHz, CD₃OD): δ 23.9, 25.0, 30.5, 35.1, 36.1, 39.1, 44.2, 112.9, 119.3, 119.4, 121.9, 122.8, 122.9, 124.5, 127.7, 130.8, 135.5, 137.1, 174.4.

3.5.4.10. 4-[1-(benzenesulfonyl)indol-3-yl]-N-(3-imidazol-1-ylpropyl)butanamide (**10**).

10 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 15/1) to afford the desired product as pale yellow oil (0.25 mmol, 40%). LC-MS (10 min): *tr* = 5.16 min, purity >95% (at 215 nm), *m/z* 451.0 [M+H]⁺. ¹H NMR (300 MHz, DMSO-*d*₆): δ 1.77-1.91 (m, 4H), 2.13 (t, *J* = 7.3 Hz, 2H), 2.63 (t, *J* = 7.6 Hz, 2H), 3.00 (dd, *J* = 12.5 and 6.5 Hz, 2H), 3.95 (t, *J* = 7.0 Hz, 2H), 6.88 (s, 1H), 7.16 (s, 1H), 7.23-7.28 (m, 1H), 7.31-7.37 (m, 1H), 7.53-7.68 (m, 6H), 7.86-7.96 (m, 4H).

3.5.4.11. 4-[1-(2-fluoro-benzenesulfonyl)-1*H*-indol-3-yl]-N-(3-imidazol-1-yl-propyl)-butyramide (**11**).

11 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as a white solid (0.37 mmol, 57%). LC-MS: *tr* = 2.43 min, 99% purity (at 215 nm), *m/z* 466.9 [M-H]⁻, 513.0 [M-H+2Na]⁻, 468.9 [M+H]⁺. HRMS *m/z* calculated for C₂₄H₂₆FN₄O₃S [M + H]⁺ 469.1710, found 469,1712. ¹H NMR (300 MHz, CD₃OD): δ 1.98 (4H, m), 2.27 (2H, t, *J* = 7.5 Hz), 2.72 (2H, t, *J* = 7.5 Hz), 3.16 (2H, t, *J* = 6.9 Hz), 4.02 (2H, t, *J* = 6.9 Hz), 6.96 (1H, s), 7.11 (1H, s), 7.14-7.25 (3H, m), 7.30 (1H, t, *J* = 8.1 Hz), 7.46 (1H, s), 7.52-7.61 (2H, m), 7.65 (1H, s), 7.79 (1H, d, *J* = 8.4 Hz), 8.02 (1H, d, *J* = 7.8 Hz). ¹³C NMR (75 MHz, CD₃OD): δ 23.8, 24.9, 30.5, 35.1, 36.1, 44.2, 113.1, 117.0, 117.3, 119.4, 122.0, 123.1, 124.3, 124.58, 124.64, 125.7, 125.9, 127.7, 130.0, 130.8, 134.9, 136.5, 136.6, 157.1, 160.6, 174.5.

3.5.4.12. 4-[1-(3-Fluoro-benzenesulfonyl)-1*H*-indol-3-yl]-N-(3-imidazol-1-yl-propyl)-butyramide (**12**).

12 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as pale yellow white solid (0.34 mmol, 56%). LC-MS: *tr* = 2.50 min, >99% purity (at 215 nm), *m/z* 466.9 [M-H]⁻, 513.0 [M-H+2Na]⁻, 468.9 [M+H]⁺. HRMS *m/z* calculated for C₂₄H₂₆FN₄O₃S [M + H]⁺ 469.1710, found 469,1712. ¹H NMR (300 MHz, CD₃OD): δ 1.96 (4H, m), 2.24 (2H, t, *J* = 7.5 Hz), 2.72 (2H, t, *J* = 7.5 Hz), 3.16 (2H, t, *J* = 6.9 Hz), 4.03 (2H, t, *J* = 6.9 Hz), 6.96 (1H, s), 7.13 (1H, s), 7.24 (1H, t, *J* = 7.2 Hz), 7.33 (2H, m), 7.46 (1H, s), 7.53 (2H, m), 7.66 (2H, m), 7.70 (1H, d, *J* = 7.8 Hz), 7.98 (1H, d, *J* = 8.4 Hz). ¹³C NMR (75 MHz, CD₃OD): δ 23.8, 24.9, 30.5, 35.0, 36.1, 44.2, 113.4, 113.7, 119.4, 120.7, 120.9, 122.5, 122.6, 122.9, 123.3, 123.5, 124.6, 127.7, 131.1, 131.2, 131.3, 135.4, 139.7, 174.5.

3.5.4.13. 4-[1-(4-Fluorobenzenesulfonyl)-1*H*-indol-3-yl]-N-(3-imidazol-1-yl-propyl)-butyramide (**13**).

13 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as pale yellow solid (0.38 mmol, 63%). LCMS: *tr* = 2.70 min, purity > 97% (at 215 nm), *m/z* 466.9 [M-H]⁻, 512.9 [M-H+2Na]⁻, 468.8 [M+H]⁺. HRMS *m/z* calculated for C₂₄H₂₆FN₄O₃S [M + H]⁺ 469.1710, found 469,1705. ¹H NMR (CD₃OD, 300 MHz): δ 1.96 (4H, m), 2.24 (2H, t, *J* = 7.5 Hz), 2.72 (2H, t, *J* = 7.5 Hz), 3.16 (2H, t, *J* = 6.9 Hz), 4.04 (2H, t, *J* = 6.9 Hz), 6.97 (1H, s), 7.14 (1H, s), 7.15~7.35 (4H, m), 7.46 (1H, s), 7.56 (1H, d, *J* = 8.4 Hz), 7.67 (1H, s), 7.97 (3H, m). ¹³C NMR (75 MHz, CD₃OD): δ 25.2, 26.3, 31.9, 36.4, 37.4, 45.6, 114.8, 115.5, 117.5 (d, *J* = 23.4 Hz; 2C), 120.7, 124.3, 124.5, 124.6, 125.9, 129.7, 130.2, 131.0 (d, *J* = 9.9 Hz, 2C), 132.4, 135.4 (d, *J* = 3.0 Hz; 1C), 136.7, 167.1 (d, *J* = 254.7 Hz; 1C), 175.8

3.5.4.14. 4-[1-(4-Chlorobenzenesulfonyl)-1*H*-indol-3-yl]-*N*-(3-imidazol-1-yl-propyl)-butyramide (14**).**

14 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as a colorless viscous oil (0.46 mmol, 77%). LC-MS (10 min): *tr* = 5.58 min, purity 95% (at 215 nm), *m/z* 482.8 [M-H]⁻, 528.9 [M-H+2Na]⁻, 484.8 [M+H]⁺. HRMS *m/z* calculated for C₂₄H₂₆ClN₄O₃S [M + H]⁺ 485.1414, found 485,1411. ¹H NMR (CD₃OD, 300 MHz): δ 1.98 (4H, m), 2.23 (2H, t, *J* = 7.5 Hz), 2.72 (2H, t-like), 3.15 (2H, t, *J* = 6.9 Hz), 4.03 (2H, t-like), 6.96 (1H, s), 7.13 (1H, s), 7.24 (1H, m), 7.32 (1H, m), 7.40~7.58 (4H, m), 7.66 (1H, s), 7.85 (2H, m), 7.94 (1H, d, *J* = 8.4 Hz). ¹³C NMR (75 MHz, CD₃OD): δ 25.2, 26.3, 31.9, 36.4, 37.4, 45.6, 114.8, 120.6, 120.8, 124.3, 124.6, 124.8, 126.0, 128.9, 129.5 (2C), 130.6 (2C), 132.5, 136.8, 137.8, 138.4, 141.5, 175.8.

3.5.4.15. 4-[1-(4-Bromobenzenesulfonyl)-1*H*-indol-3-yl]-*N*-(3-imidazol-1-yl-propyl)-butyramide (15**).**

15 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as a colorless viscous oil (0.45 mmol, 74%). LC-MS (10 min): *tr* = 5.57 min, purity > 99% (at 215 nm), *m/z* 526.8 and 528.8 [M-H]⁻, 572.8 and 574.8 [M-H+2Na]⁻, 528.8 and 530.7 [M+H]⁺. HRMS *m/z* calculated for C₂₄H₂₆BrN₄O₃S [M + H]⁺ 529.0909, found 529,0912. ¹H NMR (CD₃OD, 300 MHz): δ 1.96 (4H, m), 2.23 (2H, t, *J* = 7.5 Hz), 2.72 (2H, t, *J* = 7.5 Hz), 3.15 (2H, t, *J* = 6.9 Hz), 4.04 (2H, t, *J* = 6.9 Hz), 6.97 (1H, s), 7.13 (1H, s), 7.25 (1H, t, *J* = 7.5 Hz), 7.33 (1H, t, *J* = 7.2 Hz), 7.44 (1H, s), 7.53 (1H, d, *J* = 7.5 Hz), 7.62 (2H, d, *J* = 8.7 Hz), 7.66 (1H, s), 7.77 (2H, d, *J* = 8.7 Hz), 7.94 (1H, d, *J* = 8.1 Hz). ¹³C

NMR (75 MHz, CD₃OD): δ 25.2, 26.2, 31.9, 36.4, 37.4, 45.6, 114.8, 120.7, 120.8, 124.3, 124.6, 124.9, 126.0, 128.9, 129.5 (2C), 129.9, 132.5, 133.7 (2C), 136.8, 138.2, 138.4, 175.8.

3.5.4.16. *N*-(3-imidazol-1-yl-propyl)-4-[1-(toluene-4-sulfonyl)-1*H*-indol-3-yl]-butyramide (16).

16 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as a colorless viscous oil (0.64 mmol, 62%). LC-MS (10 min): *tr* = 4.92 min, purity >99% (at 215 nm), *m/z* 463.0 [M-H]⁻, 508.9 [M-H+2Na]⁻, 464.9 [M+H]⁺. HRMS *m/z* calculated for C₂₅H₂₉N₄O₃S [M + H]⁺ 465.1960, found 465,1968. ¹H NMR (CD₃OD, 300 MHz): δ 1.96 (4H, m), 2.24 (2H, t, *J* = 7.5 Hz), 2.33 (1H, s), 2.72 (2H, m), 3.16 (2H, t, *J* = 6.9 Hz), 4.04 (2H, m), 6.96 (1H, s), 7.13 (1H, s), 7.16~7.34 (4H, m), 7.43 (1H, s), 7.54 (1H, m), 7.66 (1H, s), 7.75 (2H, m), 7.96 (1H, d, *J* = 8.4 Hz). ¹³C NMR (75 MHz, CD₃OD): δ 21.4, 25.2, 26.3, 31.9, 36.5, 37.4, 45.6, 114.8, 120.6, 124.1, 124.3, 125.7, 127.8 (2C), 129.0, 130.9 (2C), 132.3, 136.3, 136.8, 138.4, 146.5.

3.5.4.17. 4-[1-(2-Methylbenzenesulfonyl)-1*H*-indol-3-yl]-*N*-(3-imidazol-1-yl-propyl)-butyramide (17).

17 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as a pale-yellow solid (0.38 mmol, 62%). LC-MS: *tr* = 2.50 min, 99% purity (at 215 nm), *m/z* 463.0 [M-H]⁻, 508.9 [M-H+2Na]⁻, 464.9 [M+H]⁺. HRMS *m/z* calculated for C₂₅H₂₉N₄O₃S [M + H]⁺ 465.1960, found 465,1957. ¹H NMR (300 MHz, CD₃OD): δ 1.97 (4H, m), 2.27 (2H, t, *J* = 7.5 Hz), 2.48 (3H, s), 2.75 (2H, t, *J* = 7.5 Hz), 3.16 (2H, t, *J* = 6.9 Hz), 4.02 (2H, t, *J* = 6.9 Hz), 6.96 (1H, s), 7.12 (1H, s), 7.20 (2H, m), 7.29 (2H, m), 7.46 (1H, t, *J* = 7.5 Hz), 7.51 (1H, s), 7.58 (1H, m), 7.66 (2H, m), 7.80 (1H, d, *J* = 8.1 Hz). ¹³C NMR (75 MHz, CD₃OD): δ 19.0, 23.8, 25.1, 30.5, 35.1, 36.1, 44.2, 112.9, 119.4, 121.1, 122.7, 123.4, 124.2, 126.3, 127.7, 128.6, 130.5, 132.7, 133.7, 135.2, 137.3, 137.7, 174.5.

3.5.4.18. 4-[1-(4-methoxy-benzenesulfonyl)-1*H*-indol-3-yl]-*N*-(3-imidazol-1-yl-propyl)-butyramide (18).

18 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as a pale-yellow oil (0.47 mmol, 72%). LC-MS: *tr* = 2.43 min, >99% purity (at 215 nm), *m/z* 479.0 [M-H]⁻, 524.9 [M-H+2Na]⁻, 480.9 [M+H]⁺. HRMS *m/z* calculated for C₂₅H₂₉N₄O₄S [M + H]⁺ 481.1910, found 481,1923. ¹H NMR (300 MHz, CD₃OD): δ 1.91 (4H, m), 2.23 (2H, t, *J* = 7.5 Hz), 2.67 (2H, t, *J* = 7.5 Hz), 3.14 (2H, q, *J* = 6.9 Hz), 3.58 (3H, s), 3.97 (2H, t, *J* = 6.9 Hz), 6.74 (2H, d, *J* = 9.0 Hz), 6.94 (1H, s), 7.07 (1H, s), 7.16 (1H, t, *J* = 7.5 Hz), 7.27 (1H, t, *J* = 7.6 Hz), 7.43 (1H, s), 7.48 (1H, d, *J* = 7.8

Hz), 7.62 (1H, s), 7.74 (2H, d, $J = 9.0$ Hz), 7.97 (1H, d, $J = 8.1$ Hz). ^{13}C NMR (75 MHz, CD_3OD): δ 23.9, 25.0, 30.6, 35.2, 36.1, 44.2, 54.9, 113.5, 114.1, 119.26, 119.34, 122.7, 122.96, 123.03, 124.4, 127.8, 128.8, 129.1, 131.0, 135.3, 137.1, 163.9, 174.4.

3.5.4.19. 4-[1-(thiophene-3-sulfonyl)-1H-indol-3-yl]-N-(3-imidazol-1-yl-propyl)-butyramide (19).

19 was synthesized according to the general procedure A from 4-(1H-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as a white solid (0.26 mmol, 39%). LC-MS: $t_r = 2.25$ min, 100% purity (at 215 nm), m/z 454.9 $[\text{M}-\text{H}]^-$, 500.9 $[\text{M}-\text{H}+2\text{Na}]^-$, 456.9 $[\text{M}+\text{H}]^+$. HRMS m/z calculated for $\text{C}_{22}\text{H}_{25}\text{N}_4\text{O}_3\text{S}_2$ $[\text{M} + \text{H}]^+$ 457.1368, found 457,1355. ^1H NMR (300 MHz, CD_3OD): δ 1.99 (4H, m), 2.26 (2H, t, $J = 7.5$ Hz), 2.73 (2H, t, $J = 7.5$ Hz), 3.17 (2H, t, $J = 6.9$ Hz), 4.04 (2H, t, $J = 6.9$ Hz), 6.98 (1H, s), 7.14 (1H, s), 7.28 (3H, m), 7.44 (1H, s), 7.48 (1H, dd, $J = 3.0, 5.1$ Hz), 7.57 (1H, d, $J = 7.5$ Hz), 7.67 (1H, s), 7.99 (1H, d, $J = 8.1$ Hz), 8.30 (1H, s). ^{13}C NMR (75 MHz, CD_3OD): δ 23.8, 24.9, 30.5, 35.0, 36.0, 44.2, 113.4, 119.2, 119.3, 122.8, 122.9, 123.0, 124.4, 124.5, 127.7, 128.5, 131.0, 131.9, 135.4, 137.1, 137.5, 174.5.

3.5.4.20. 4-[1-(1-methyl-1H-imidazole-4-sulfonyl)-1H-indol-3-yl]-N-(3-imidazol-1-yl-propyl)-butyramide (20).

20 was synthesized according to the general procedure A from 4-(1H-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as a colorless viscous oil (0.23 mmol, 34%). LC-MS: $t_r = 1.90$ min, >99% purity (at 215 nm), m/z 453.0 $[\text{M}-\text{H}]^-$, 498.9 $[\text{M}-\text{H}+2\text{Na}]^-$, 454.9 $[\text{M}+\text{H}]^+$. HRMS m/z calculated for $\text{C}_{22}\text{H}_{27}\text{N}_6\text{O}_3\text{S}$ $[\text{M} + \text{H}]^+$ 455.1865, found 455,1858. ^1H NMR (300 MHz, CD_2Cl_2): δ 2.00 (4H, quint, $J = 6.8$ Hz), 2.16 (2H, t, $J = 6.9$ Hz), 2.73 (2H, t, $J = 6.9$ Hz), 3.24 (2H, q, $J = 6.4$ Hz), 3.62 (3H, s), 4.02 (2H, t, $J = 6.9$ Hz), 6.98 (1H, s), 7.01 (1H, s), 7.24 (1H, t, $J = 7.5$ Hz), 7.32 (1H, t, $J = 7.5$ Hz), 7.38 (1H, s), 7.40 (1H, s), 7.52 (2H, s), 7.54 (1H, brs), 7.69 (1H, s), 7.93 (1H, d, $J = 6.9$ Hz). ^{13}C NMR (75 MHz, CD_2Cl_2): δ 23.8, 24.3, 31.1, 34.1, 34.9, 36.3, 44.6, 113.8, 119.2, 119.6, 121.9, 123.1, 124.1, 124.5, 125.4, 128.6, 131.0, 135.4, 137.2, 137.5, 139.8, 146.6, 173.0.

3.5.4.21. 4-[1-(3,5-dimethyl-isoxazole-4-sulfonyl)-1H-indol-3-yl]-N-(3-imidazol-1-yl-propyl)-butyramide (21).

21 was synthesized according to the general procedure A from 4-(1H-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as a colorless viscous oil (0.17 mmol, 18%). LC-MS: $t_r = 2.27$ min, 100% purity (at 215 nm), m/z

467.9 [M-H]⁻, 514.0 [M-H+2Na]⁻, 469.9 [M+H]⁺. HRMS *m/z* calculated for C₂₃H₂₈N₅O₄S [M + H]⁺ 470.1862, found 470,1856. ¹H NMR (300 MHz, CD₂Cl₂): δ 2.00 (2H, quint, *J* = 6.9 Hz), 2.06 (2H, quint, *J* = 7.5 Hz), 2.25 (2H, t, *J* = 7.5 Hz), 2.29 (3H, s), 2.74 (3H, s), 2.76 (2H, t, *J* = 7.5 Hz), 3.24 (2H, q, *J* = 6.4 Hz), 3.99 (2H, t, *J* = 6.9 Hz), 6.56 (1H, brs), 6.99 (2H, s), 7.32 (1H, t, *J* = 7.5 Hz), 7.36 (1H, s), 7.37 (1H, t, *J* = 7.5 Hz), 7.45 (1H, s), 7.60 (1H, d, *J* = 7.5 Hz), 7.85 (1H, d, *J* = 7.8 Hz). ¹³C NMR (75 MHz, CD₂Cl₂): δ 10.9, 13.1, 24.2, 24.8, 31.2, 35.6, 36.4, 44.6, 113.3, 115.6, 119.0, 120.0, 122.6, 123.0, 123.6, 124.9, 128.9, 131.2, 135.0, 137.1, 137.4, 157.4, 172.6, 174.6.

3.5.4.22. 4-[1-(pyridine-3-sulfonyl)-1*H*-indol-3-yl]-butyric acid -*N*-(3-imidazol-1-yl-propyl)-butyramide (22**).**

22 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as a beige solid (0.18 mmol, 24%). LC-MS: *tr* = 2.02 min, 100% purity (at 215 nm), *m/z* 450.1 [M-H]⁻, 496.0 [M-H+2Na]⁻, 451.9 [M+H]⁺. HRMS *m/z* calculated for C₂₃H₂₆N₅O₃S [M + H]⁺ 452.1756, found 452,1766. ¹H NMR (300 MHz, CD₃OD): δ 1.99 (4H, m), 2.24 (2H, t, *J* = 7.5 Hz), 2.73 (2H, t, *J* = 7.5 Hz), 3.17 (2H, t, *J* = 6.9 Hz), 4.05 (2H, t, *J* = 6.9 Hz), 6.98 (1H, s), 7.15 (1H, s), 7.27 (1H, t, *J* = 7.5 Hz), 7.36 (1H, t, *J* = 7.8 Hz), 7.51 (1H, s), 7.52 (1H, t, *J* = 6.6 Hz), 7.57 (1H, d, *J* = 7.2 Hz), 7.68 (1H, s), 8.03 (1H, d, *J* = 8.4 Hz), 8.29 (1H, d, *J* = 8.1 Hz), , 8.72 (1H, d, *J* = 4.8 Hz), 9.06 (1H, s). ¹³C NMR (75 MHz, CD₃OD): δ 23.8, 24.8, 30.5, 35.0, 36.0, 44.2, 113.4, 119.2, 119.5, 122.7, 123.5, 124.1, 124.2, 124.9, 127.7, 131.2, 134.6, 134.8, 135.3, 137.1, 146.7, 153.9, 174.4.

3.5.4.23. 4-[1-(benzo[1,2,5]thiadiazole-4-sulfonyl)-1*H*-indol-3-yl]-*N*-(3-imidazol-1-yl-propyl)-butyramide (23**).**

23 was synthesized according to the general procedure A from 4-(1*H*-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 15/1) to afford the desired product as a yellow viscous oil (0.33 mmol, 42%). LC-MS: *tr* = 2.27 min, 100% purity (at 215 nm), *m/z* 553.0 [M-H+2Na]⁻, 508.9 [M+H]⁺. HRMS *m/z* calculated for C₂₄H₂₅N₆O₃S₂ [M + H]⁺ 509.1430, found 509,1422. ¹H NMR (300 MHz, CD₂Cl₂): δ 2.01 (4H, m), 2.27 (2H, t, *J* = 7.5 Hz), 2.72 (2H, t, *J* = 7.2 Hz), 3.24 (2H, q, *J* = 6.4 Hz), 4.00 (2H, t, *J* = 7.2 Hz), 6.97 (1H, s), 6.99 (1H, s), 7.04 (1H, brs), 7.18 (1H, t, *J* = 7.5 Hz), 7.27 (1H, t, *J* = 7.6 Hz), 7.46 (1H, s), 7.48 (1H, d, *J* = 6.9 Hz), 7.72 (1H, dd, *J* = 7.2, 9.0 Hz), 7.82 (1H, s), 7.93 (1H, d, *J* = 7.8 Hz), 8.17 (1H, d, *J* = 9.0 Hz), 8.45 (1H, d, *J* = 7.2 Hz). ¹³C NMR (75 MHz, CD₂Cl₂): δ 24.2, 24.9, 31.2, 35.6, 36.4, 44.6, 113.3, 119.1, 119.6, 121.3, 123.1, 124.4, 124.7, 127.7, 127.9, 128.8, 129.5, 131.0, 131.9, 134.7, 137.1, 148.8, 155.5, 172.9.

3.5.4.24. 4-[1-(biphenyl-4-sulfonyl)-1H-indol-3-yl]-N-(3-imidazol-1-yl-propyl)-butyramide (**24**).

24 was synthesized according to the general procedure A from 4-(1H-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 25/1) to afford the desired product as colorless viscous oil (0.32 mmol, 71%). LC-MS: tr = 2.70 min, 100% purity (at 215 nm), *m/z* 525.0 [M-H]⁻, 570.9 [M-H+2Na]⁻, 526.9 [M+H]⁺. HRMS *m/z* calculated for C₃₀H₃₁N₄O₃S [M + H]⁺ 527.2117, found 527,2122. ¹H NMR (300 MHz, CD₂Cl₂): δ 1.94 (2H, quint, *J* = 6.6 Hz), 2.05 (2H, quint, *J* = 7.5 Hz), 2.26 (2H, t, *J* = 7.5 Hz), 2.74 (2H, t, *J* = 7.5 Hz), 3.22 (2H, q, *J* = 6.4 Hz), 3.98 (2H, t, *J* = 6.9 Hz), 6.67 (1H, brs), 7.00 (1H, d, *J* = 3.6 Hz), 7.28 (1H, t, *J* = 7.5 Hz), 7.36-7.57 (9H, m), 7.65 (2H, d, *J* = 6.6 Hz), 7.93 (2H, d, *J* = 6.9 Hz), 8.08 (2H, d, *J* = 8.4 Hz). ¹³C NMR (75 MHz, CD₂Cl₂): δ 24.3, 24.8, 31.2, 35.6, 36.4, 44.6, 113.7, 119.1, 119.7, 122.9, 123.1, 123.2, 124.8, 127.2, 127.8, 128.7, 128.9, 129.0, 131.1, 135.3, 136.4, 137.1, 138.7, 146.6, 172.8.

3.5.4.25. 4-[1-(naphthalene-1-sulfonyl)-1H-indol-3-yl]-N-(3-imidazol-1-yl-propyl)-butyramide (**25**).

25 was synthesized according to the general procedure A from 4-(1H-indol-3-yl)-butyric acid and it was purified by flash column chromatography on silica gel (DCM/MeOH, 22/1) to afford the desired product as white solid (0.29 mmol, 53%). LC-MS: tr = 2.48 min, 100% purity (at 215 nm), *m/z* 499.1 [M-H]⁻, 545.0 [M-H+2Na]⁻, 500.9 [M+H]⁺. HRMS *m/z* calculated for C₂₈H₂₉N₄O₃S [M + H]⁺ 501.1960, found 501,1960. ¹H NMR (300 MHz, CD₂Cl₂): δ 1.95 (2H, quint, *J* = 6.9 Hz), 2.02 (2H, quint, *J* = 6.9 Hz), 2.21 (2H, t, *J* = 7.5 Hz), 2.74 (2H, t, *J* = 7.2 Hz), 3.21 (2H, q, *J* = 6.5 Hz), 3.96 (2H, t, *J* = 6.9 Hz), 6.44 (1H, brs), 7.00 (1H, d, *J* = 6.3 Hz), 7.24 (1H, quint, *J* = 7.8 Hz), 7.45 (1H, s), 7.51-7.55 (2H, m), 7.56-7.58 (1H, m), 7.62 (1H, s), 7.68 (1H, t, *J* = 7.8 Hz), 7.81 (1H, d, *J* = 7.5 Hz), 7.92 (1H, d, *J* = 7.5 Hz), 8.09 (1H, d, *J* = 8.4 Hz), 8.18 (1H, d, *J* = 7.5 Hz). ¹³C NMR (75 MHz, CD₂Cl₂): δ 24.2, 24.8, 31.2, 35.6, 36.4, 44.5, 113.2, 119.0, 119.7, 121.7, 123.0, 123.3, 123.9, 124.2, 124.6, 127.2, 128.0, 128.6, 129.0, 129.2, 129.4, 130.7, 133.8, 134.2, 135.1, 135.5, 137.1, 172.7.

3.5.4.26. 4-[1-(naphthalene-1-sulfonyl)-1H-indol-3-yl]-N-(3-imidazol-1-yl-propyl)-butyramide (**26**).

Commercial 1H-indole-3-carbaldehyde **49e** (3.35 mmol), NaOH (10 mmol) and Bu₄NHSO₄ (0.168 mmol) in dry DCM/THF 2/1 was stirred for 5 min at room temperature. Benzenesulfonyl chloride (3.85 mmol) was added. The mixture was stirred for 1 h at room temperature. It was diluted with DCM, washed with brine, dried over MgSO₄, filtered and concentrated. The precipitate was recrystallized in isopropanol to afford the desired product as grey yellow solid (2.11 mmol, 63%). LC-MS: tr = 2.78 min, *m/z* 300.1 [M-H]⁻, 301.9 [M+H]⁺. To the aldehyde (2.11 mmol) (4/1) were added sulfamic acid (6.0 eq.) and sodium

chlorite (1.5 eq.). The mixture was stirred for 1 h at room temperature. Dioxane was removed under reduced pressure. The remained aqueous residue was adjusted to pH ~ 4 with saturated NaHCO₃ solution and extracted three times with EtOAc. The combined organic layer was washed with H₂O and brine, dried over MgSO₄, filtered and concentrated. The residue was agitated to precipitate by addition of EtOAc/cyclohexane (5 ml / 30 ml). The precipitate was collected to give the crude product (**50e**) as pale solid (1.88 mmol, 89%), which was pure enough to be directly used in the next step. LC-MS: tr = 3.02 min, *m/z* 284.1 [M-H]⁻, 286.0 [M+H]⁺. To the mixture of the carboxylic acid (1.12 mmol), HOBT (1.5 eq.) and EDCI (2 eq.) in dry DMF was added DIPEA (4 eq.). After stirred for 10 min at room temperature, 1-(3-aminopropyl)imidazole (1.5 eq.) was added. The mixture was stirred overnight at room temperature. DMF was under reduced pressure. The residue was diluted with DCM, washed successively twice with saturated NaHCO₃ aqueous solution, brine, dried over MgSO₄, filtered and concentrated. The residue was purified by flash column chromatography over silica gel (DCM/MeOH, 15/1) and preparative LCMS to afford the desired product as a pale-yellow viscous oil (0.33 mmol, 30%). LCMS (10 min): tr = 2.40 min, purity 98% (at 215 nm), *m/z* 407.0 [M-H]⁻, 453.0 [M+2Na-H]⁻, 408.9 [M+H]⁺. HRMS *m/z* calculated for C₂₁H₂₁N₄O₃S [M + H]⁺ 409.1334, found 409,1336. ¹H NMR (CD₂Cl₂, 300 MHz): δ 2.14 (2H, quint, *J* = 6.6 Hz), 3.44 (2H, q, *J* = 6.0 Hz), 4.11 (2H, t, *J* = 6.6 Hz), 7.08 (2H, s), 7.29~7.59 (6H, m), 7.85 (1H, s), 7.90 (2H, d, *J* = 7.5 Hz), 7.96 (1H, d, *J* = 8.1 Hz), 8.14 (1H, d, *J* = 7.5 Hz), 8.26 (1H, s). ¹³C NMR (CD₂Cl₂, 75 MHz): δ 31.4, 36.9, 45.4, 113.6, 117.8, 119.8, 122.5, 124.5, 125.7, 127.3 (2C), 128.3, 128.3, 129.9 (2C), 134.8, 135.1, 137.4, 137.8.

3.5.4.27. 2-(1-Benzenesulfonyl-1H-indol-3-yl)-N-(3-imidazol-1-yl-propyl)-acetamide (**27**).

27 was synthesized according to the general procedure A and it was purified by flash column chromatography on silica gel (DCM/MeOH, 18/1) to afford the desired product as white solid (0.58 mmol, 72%). LC-MS (10 min): tr = 4.62 min, purity 100% (at 215 nm), *m/z* 421.0 [M-H]⁻, 467.0 [M+2Na-H]⁻, 422.9 [M+H]⁺. HRMS *m/z* calculated for C₂₂H₂₃N₄O₃S [M + H]⁺ 423.1491, found 423,1497. ¹H NMR (CDCl₃, 300 MHz): δ 1.87 (2H, quint, *J* = 6.9 Hz), 3.16 (2H, q, *J* = 6.6 Hz), 3.62 (2H, s), 3.82 (2H, t, *J* = 6.9 Hz), 5.94 (1H, brs), 6.79 (1H, s), 7.00 (1H, s), 7.30 (2H, d, *J* = 8.4 Hz), 7.35~7.57 (6H, m), 7.89 (2H, d, *J* = 7.5 Hz), 8.00 (1H, d, *J* = 8.4 Hz). ¹³C NMR (CDCl₃, 75 MHz): δ 30.8, 33.1, 36.6, 44.4, 113.6, 116.5, 118.9, 119.7, 123.5, 124.7, 125.2, 126.7 (2C), 129.0, 129.3 (2C), 130.3, 134.0, 135.1, 136.9, 137.8.

3.5.4.28. 3-(1-Benzenesulfonyl-1H-indol-3-yl)-N-[3-imidazol-1-yl-propyl]-propionamide (**28**).

28 was synthesized according to the general procedure A and it was purified by flash column chromatography on silica gel (DCM/MeOH, 22/1) to afford the desired product as white solid (0.48 mmol, 71%). LC-MS (10 min): tr = 4.65 min, purity 100% (at 215 nm), m/z 435.0 [M-H]⁻, 481.0 [M+2Na-H]⁻, 437.0 [M+H]⁺. HRMS m/z calculated for C₂₃H₂₅N₄O₃S [M + H]⁺ 437.1647, found 437,1647. ¹H NMR (300 MHz, CDCl₃): δ 1.88 (2H, quint, J = 6.9 Hz), 2.52 (2H, t, J = 7.5 Hz), 3.02 (2H, t, J = 7.5 Hz), 3.16 (2H, q, J = 6.3 Hz), 3.83 (2H, t, J = 6.9 Hz), 6.39 (1H, brs), 6.86 (1H, s), 6.98 (1H, s), 7.22~7.43 (5H, m), 7.49 (2H, d, J = 7.2 Hz), 7.82 (2H, d, J = 7.8 Hz), 7.95 (1H, d, J = 8.4 Hz). ¹³C NMR (CDCl₃, 75 MHz): δ 20.9, 30.8, 35.8, 36.4, 44.4, 113.6, 119.0, 119.6, 122.3, 122.7, 123.3, 124.9, 126.6 (2C), 129.0, 129.3 (2C), 130.7, 133.8, 135.1, 137.0, 138.0

3.5.4.29. 5-(1-benzenesulfonyl-1H-indol-3-yl)-pentanoic acid (3-imidazol-1-yl-propyl)-amide (29).

(29) was synthesized according to the general procedure A and it was purified by flash column chromatography on silica gel (DCM/MeOH, 22/1) to afford the desired product as a pale-yellow oil (0.27 mmol, 67%). LC-MS: tr = 2.32 min, 100% purity (at 215 nm), m/z 464.0 [M-H]⁻, 510.0[M-H+2Na]⁻, 465.8 [M+H]⁺. HRMS m/z calculated for C₂₅H₂₉N₄O₃S [M + H]⁺ 465.1960, found 465,1963. ¹H NMR (300 MHz, CD₃OD): δ 1.67 (4H, m), 1.95 (2H, quint, J = 6.9 Hz), 2.22 (2H, t, J = 6.9 Hz), 2.67 (2H, t, J = 6.9 Hz), 3.17 (2H, t, J = 6.9 Hz), 4.01 (2H, t, J = 6.9 Hz), 6.97 (1H, s), 7.11 (1H, s), 7.20 (1H, t, J = 7.8 Hz), 7.30 (1H, t, J = 7.2 Hz), 7.41-7.56 (5H, m), 7.65 (1H, s), 7.86 (2H, d, J = 7.8 Hz), 7.99 (1H, d, J = 8.4 Hz). ¹³C NMR (75 MHz, CD₃OD): δ 23.9, 25.2, 28.3, 30.6, 35.4, 36.0, 44.1, 113.5, 119.2, 119.3, 122.8, 123.0, 123.5, 124.4, 126.4, 127.7, 129.0, 131.1, 133.6, 135.4, 137.8, 174.8.

3.5.4.30. 4-(1-benzenesulfonyl-1H-indol-3-yl)-N-(4-imidazol-1-yl-butyl)-butyramide (30).

30 was synthesized according to the general procedure A and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as a colorless oil (0.33 mmol, 65%). LC-MS: tr = 2.28 min, 100% purity (at 215 nm), m/z 463.1 [M-H]⁻, 509.1[M-H+2Na]⁻, 465.0 [M+H]⁺. HRMS m/z calculated for C₂₅H₂₉N₄O₃S [M + H]⁺ 465.1960, found 465,1965. ¹H NMR (300 MHz, CD₃OD): δ 1.46 (2H, quint, J = 7.2 Hz), 1.79 (2H, quint, J = 7.2 Hz), 1.98 (2H, quint, J = 7.2 Hz), 2.23 (2H, t, J = 7.2 Hz), 2.70 (2H, t, J = 7.2 Hz), 3.19 (2H, t, J = 7.2 Hz), 4.04 (2H, t, J = 7.2 Hz), 6.95 (1H, s), 7.11 (1H, s), 7.23 (1H, t, J = 7.2 Hz), 7.32 (1H, t, J = 7.5 Hz), 7.46 (1H, s), 7.47-7.61 (4H, m), 7.65 (1H, s), 7.89 (2H, d, J = 8.1 Hz), 7.99 (1H, d, J = 8.1 Hz). ¹³C NMR (75 MHz, CD₃OD): δ 23.8, 25.0, 26.1, 28.1, 35.1, 38.2, 46.1, 113.4, 119.3, 122.90, 123.0, 124.4, 126.4, 127.6, 129.0, 131.0, 133.7, 135.4, 137.9, 174.3.

3.5.4.31. 4-(1-benzenesulfonyl-1H-indol-3-yl)-N-(2-imidazol-1-yl-ethyl)-butyramide (**31**).

31 was synthesized according to the general procedure A and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as a colorless oil (0.37 mmol, 73%). LC-MS: tr = 2.20 min, > 99% purity (at 215 nm), m/z 435.1 [M-H]⁻, 481.1[M-H+2Na]⁻, 437.0 [M+H]⁺. HRMS m/z calculated for C₂₃H₂₅N₄O₃S [M + H]⁺ 437.1647, found 437,1647. ¹H NMR (300 MHz, CD₃OD): δ 1.94 (2H, quint, J = 7.2 Hz), 2.21 (2H, t, J = 7.2 Hz), 2.66 (2H, t, J = 7.5 Hz), 3.52 (2H, t, J = 6.0 Hz), 4.14 (2H, t, J = 6.0 Hz), 6.98 (1H, s), 7.13 (1H, s), 7.24 (1H, t, J = 7.8 Hz), 7.32 (1H, t, J = 8.1 Hz), 7.44 (1H, s), 7.47-7.59 (4H, m), 7.66 (1H, s), 7.89 (2H, d, J = 7.5 Hz), 7.99 (1H, d, J = 8.4 Hz). ¹³C NMR (75 MHz, CD₃OD): δ 23.8, 24.9, 34.9, 39.7, 45.7, 113.4, 119.3, 122.90, 122.94, 123.0, 124.4, 126.4, 127.5, 129.0, 130.9, 133.7, 135.4, 137.9, 174.7.

3.5.4.32. 4-(1-benzenesulfonyl-1H-indol-3-yl)-N-(3-imidazol-1-yl-propyl)-N-methyl-butylamide (**32**).

32 was synthesized according to the general procedure A and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as a sticky colorless oil (0.18 mmol, 35%). LC-MS: tr = 2.32 min, 97% purity (at 215 nm), m/z 509.1 [M-H+2Na]⁻, 465.0 [M+H]⁺. HRMS m/z calculated for C₂₅H₂₉N₄O₃S [M + H]⁺ 465.1960, found 465,1974. ¹H NMR (300 MHz, CD₃OD): δ 1.97 (4H, m), 2.34 (2.22) (2H, t, J = 7.2 Hz), 2.71 (2H, m), 2.87 (3H, s), 3.35 (3.19) (2H, t, J = 6.9 Hz), 4.02 (3.92) (2H, t, J = 6.9 Hz), 7.07 (1H, s), 7.21 (7.10) (1H, t, J = 7.2 Hz), 7.23 (1H, s), 7.30 (1H, t, J = 7.5 Hz), 7.35-7.57 (5H, m), 7.80-8.10 (4H, m). ¹³C NMR (75 MHz, CD₃OD): δ 23.8 (23.6), 24.2 (24.5), 28.1 (29.0), 32.1 (31.4), 34.4 (32.4), 44.6 (44.0), 44.8, 113.5, 119.4, 119.7, 122.9, 123.1, 123.2, 124.5, 126.2 (127.3), 126.4, 129.0, 131.0, 133.7, 135.4, 136.7 (136.9), 137.9, 173.9 (173.4).

3.5.4.33. N-[3-(1-Benzenesulfonyl-1H-indol-3-yl)-propyl]-4-imidazol-1-yl-butylamide (**33**).

To a solution of the commercial indolebutyric acid (**35a**) in MeOH in MeOH was dropwise added H₂SO₄ (3.3 eq.) with controlled temperature below 45 °C. The mixture was stirred at ambient temperature for 2 h. It was poured into H₂O, adjusted to pH ~ 8 with NaHCO₃ and extracted with EtOAc. The combined organic layer was washed with H₂O and brine, dried over MgSO₄, filtered and concentrated to give the methyl ester intermediate. The crude product was then solubilized in DCM and NaOH (3.0 eq.) and Bu₄NHSO₄ (0.05 eq.) were added and left to stir 10 min at room temperature before the sulfonyl chloride derivative (1.05 eq.) was added and left to stir overnight at room temperature. The solid was filtered off. The filtrate was concentrated and dissolved in MeOH (8 ml) / THF (4 ml). Aqueous solution of NaOH (120 mg, 3mmol) in H₂O (3 ml) was added. The mixture was stirred at room

temperature for 3 h. It was diluted with H₂O and adjusted to pH ~ 4 with 1M KHSO₄ solution. The precipitate was collected with filter, washed with H₂O, and dried in a vacuum desiccator to afford the desired product 3-(1-benzenesulfonyl-1*H*-indol-3-yl)-propionamide as white solid (98% yield). The carboxylic acid was treated with PyBOP (1.5 eq.), HOBt (1.5 eq.), DIPEA (4 eq.), NH₄Cl (2 eq.) in DMF and left to stir at room temperature overnight. DMF was removed under reduced pressure and the residue was diluted with DCM, successively washed twice with aqueous saturated NaHCO₃ solution, brine, dried over MgSO₄, filtered and concentrated under reduced pressure to give a brown oil. Cyclohexane was added and evaporated off. The precipitate was collected by a filter and recrystallized in isopropanol/H₂O (8 ml /15 ml) to afford the desired product as white solid corresponding unsubstituted amide **35** (1.14 mmol, 71 %), LC-MS: tr = 2.68 min, purity 96% (at 215 nm), *m/z* 327.0 [M-H]⁻, 373.0 [M+2Na-H]⁻, 329.0 [M+H]⁺. HRMS *m/z* calculated for C₁₇H₁₇N₂O₃S [M + H]⁺ 329.0960, found 329,0959. ¹H NMR (CD₃OD, 300 MHz): δ 2.58 (2H, t, *J* = 7.8 Hz), 2.99 (2H, t, *J* = 7.8 Hz), 7.24 (1H, t, *J* = 7.8 Hz), 7.31 (1H, t, *J* = 7.8 Hz), 7.47 (3H, m), 7.57 (1H, d, *J* = 7.5 Hz), 7.86 (2H, d, *J* = 7.2 Hz), 7.94 (1H, d, *J* = 8.1 Hz). ¹³C NMR (CD₃OD, 75 MHz): δ 21.6, 35.8, 114.7, 120.5, 123.8, 124.4 (2C), 125.8, 127.8 (2C), 130.3 (2C), 132.2, 135.0, 136.7, 139.2. **35** was solubilized in DME and AlCl₃ (8.0 eq.) was added and the solution was left to stir 30 min at room temperature. NaBH₄ (8.0 eq.) was added by portion and the mixture was left to stir 40h at room temperature. It was carefully quenched with H₂O (30 ml), basified to pH ~9 with NaOH solution (1 M) and extracted three times with EtOAc. The combined organic layer was washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography over silica gel (DCM/MeOH = 10/1, containing 0.2% TEA,) to give **36** as white solid (0.86 mmol, 45%). LC-MS: tr = 2.47 min, purity > 98% (at 215 nm), *m/z* 313.1 [M-H]⁻, 315.0 [M+H]⁺. HRMS *m/z* calculated for C₁₇H₁₉N₂O₂S [M + H]⁺ 315.1167, found 315,1167. ¹H NMR (CD₃OD, 300 MHz): δ 1.80 (2H, t, *J* = 7.5 Hz), 2.65 (4H, m), 7.21 (1H, t, *J* = 7.2 Hz), 7.29 (1H, t, *J* = 7.2 Hz), 7.41 (3H, m), 7.50 (2H, m), 7.86 (2H, d, *J* = 7.2 Hz), 7.96 (1H, d, *J* = 8.1 Hz). ¹³CNMR (CD₃OD, 300 MHz): δ 21.7, 31.7, 40.7, 113.5, 119.3, 122.8, 123.0, 123.5, 124.4, 126.4, 129.0, 131.1, 133.6, 135.5, 137.8. The acid **33b** was obtained from ethyl 4-bromobutanate and imidazole in a 2-step reaction, first with NaH (1.0 eq.) in DMF at 80°C for 1h. DMF was removed by rotary evaporation in vacuo. The residue was diluted with EtOAc, washed with H₂O dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (EtOAc/MeOH, 8/1) to afford the intermediate as a colorless oil (4.01mmol, 17%). The mixture of the

intermediate methyl ester (4.18mmol), NaOH (1.2 eq.), H₂O and EtOH was stirred at room temperature for 30 min. The mixture was adjusted to pH ~ 5 with HCl solution (1 M). The solvents were removed under reduced pressure. The residue was solubilized with hot EtOH and filtered. The filtrate was concentrated to afford the desired product **33b** a colorless oil. (4.01 mmol, 96%). ¹H NMR (D₂O, 300 MHz): δ 2.02 (2H, quint, *J* = 6.9 Hz), 2.16 (2H, t, *J* = 6.9 Hz), 4.14 (2H, t, *J* = 6.9 Hz), 7.33 (1H, s), 7.40 (1H, s), 8.61 (1H, s). The data is identical to that in lit. J. Org. Chem., 2004, 69 (21): 7171. To the mixture of the acid **33b**, HOBt (1.5 eq.) and EDCI (2.5 eq.) in DMF was added DIPEA (4.0 eq.). After being stirred for 10 min at room temperature, the amine **36** (1.05 eq.) was added and the mixture was left to stir overnight at room temperature. DMF was removed under reduced pressure and the residue was diluted with DCM, successively washed twice with aqueous saturated NaHCO₃ solution, brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was to afford the desired product **33**. It was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product **33** as a pale-yellow viscous oil (0.60 mmol, 52%). LC-MS: t_r = 2.53 min, purity 100% (at 215 nm), *m/z* 449.0 [M-H]⁻, 494.9 [M+2Na-H]⁻, 450.9 [M+H]⁺. HRMS *m/z* calculated for C₂₄H₂₇N₄O₃S [M + H]⁺ 451.1804, found 451,1797. ¹H NMR (CD₃OD, 300 MHz): δ 1.83 (2H, quint, *J* = 7.5 Hz), 2.04 (2H, quint, *J* = 6.9 Hz), 2.16 (2H, t, *J* = 6.9 Hz), 2.65 (2H, t, *J* = 7.5 Hz), 3.19 (2H, t, *J* = 7.2 Hz), 3.99 (2H, t, *J* = 7.2 Hz), 6.96 (1H, s), 7.08 (1H, s), 7.17 (1H, t, *J* = 7.2 Hz), 7.27 (1H, t, *J* = 7.2 Hz), 7.36 (2H, m), 7.44 (2H, m), 7.46 (1H, s), 7.62 (1H, s), 7.85 (2H, d, *J* = 7.2 Hz), 7.95 (1H, d, *J* = 8.4 Hz). ¹³CNMR (CD₃OD, 300 MHz): δ 21.7, 26.8, 28.3, 32.1, 38.6, 46.0, 113.5, 119.2, 119.3, 112.9, 123.1, 124.5, 126.5, 127.8, 129.0, 131.0, 133.7, 135.4, 137.1, 137.8, 173.2.

3.5.4.34. 1-[2-(1-Benzenesulfonyl-1H-indol-3-yl)-ethyl]-3-(3-imidazol-1-yl-propyl)-urea (**34**).

A mixture of commercial N-Boc-tryptamine **34a**, NaOH (3.0 eq.) and Bu₄NHSO₄ (0.05 eq.) in dry DCM was stirred for 5~10min before benzenesulfonyl chloride (1.05 eq.) was added. The mixture was stirred for 3h at room temperature. It was diluted with DCM, filtered and concentrated under reduced pressure. The residue was dissolved in HCl 4M in dioxane and left to stir overnight at room temperature. Water was added adjusted to basic pH with NH₄OH and extracted with EtOAc. The extracted organic layer was washed with brine, dried over MgSO₄, and concentrated under reduced pressure to afford the desired product **34b** as light brown oil, which became waxy residue (1.78 mmol, 96%). ¹H NMR (CDCl₃, 300 MHz): δ 1.88 (2H, brs), 2.76 (2H, t, *J* = 6.8 Hz), 2.94 (2H, t, *J* = 6.8 Hz), 7.16 (1H, t, *J* = 7.5 Hz), 7.21~7.41 (5H, m), 7.46 (1H, d, *J* = 7.8 Hz), 7.82 (2H, d, *J* = 7.8 Hz), 7.97 (1H, d, *J* = 8.4 Hz). To **34b**

(136 mg, 0.45 mmol) dissolved in THF was added 4-nitrophenyl chloroformate (1.1 eq.) and left to stir for 1h at room temperature. Triethylamine (632 μ l, 4.5 mmol) was added, followed by 3-imidazol-1-yl-propylamine (2.0 eq.) and left to stir for an additional 3h. Solids were filtered off and the filtrate was concentrated under reduced pressure. The residue was diluted with DCM, washed twice with saturated NaHCO₃ solution, brine, dried over MgSO₄, filtered and concentrated under reduced pressure. It was purified by flash column chromatography on silica gel (DCM/MeOH, 15/1) to afford the desired product **34** as a pale-yellow sticky oil (0.33 mmol, 73%). LC-MS: tr = 2.79 min, 100% purity (at 215 nm), *m/z* 496.4 [M-H+2Na]⁻, 452.3 [M+H]⁺. HRMS *m/z* calculated for C₂₃H₂₆N₅O₃S [M + H]⁺ 452.1756, found 452,1764. ¹H NMR (300 MHz, CD₃OD): δ 1.89 (2H, quint, *J* = 6.9 Hz), 2.85 (2H, t, *J* = 7.2 Hz), 3.08 (2H, t, *J* = 6.6 Hz), 3.42 (2H, t, *J* = 7.2 Hz), 3.98 (2H, t, *J* = 7.2 Hz), 6.97 (1H, s), 7.10 (1H, s), 7.20 (1H, t, *J* = 7.8 Hz), 7.28 (1H, t, *J* = 7.2 Hz), 7.42 (2H, m), 7.51 (3H, m), 7.65 (1H, s), 7.86 (2H, d, *J* = 7.2 Hz), 7.96 (1H, d, *J* = 8.1 Hz). ¹³C NMR (75 MHz, CD₃OD): δ 25.3, 31.4, 36.5, 39.3, 44.1, 113.3, 119.27, 119.32, 120.7, 123.0, 123.4, 124.4, 126.4, 127.5, 129.0, 131.0, 133.7, 135.2, 137.0, 137.9, 159.6.

3.5.4.35. 3-(1-Benzenesulfonyl-1H-indol-3-yl)-propylamine (35). Synthesis of **35** is described in paragraph 4.5.4.34 as it is an intermediate for **33**.

3.5.4.36. 3-(1-Benzenesulfonyl-1H-indol-3-yl)-propionamide (36). Synthesis of **35** is described in paragraph 4.5.4.34 as it is an intermediate for **33**.

3.5.4.37. 1-Benzenesulfonyl-N-Boc-L-tryptophan (37S).

37S was synthesized according to the general procedure B: product was obtained as a light-yellow solid (2.13 mmol, 85%) LC-MS: tr = 3.03 min, purity 96% (at 215 nm), *m/z* 442.9 [M-H]⁻, 388.8 [M-C₄H₉+H]⁺, 345.0 [M-Boc+H]⁺. ¹H NMR (CD₃OD, 300 MHz): δ 1.38 (9H, s), 3.04 (1H, m), 3.26 (1H, m), 4.42 (1H, m), 7.21~7.32 (2H, m), 7.44~7.58 (5H, m), 7.85 (2H, d, *J* = 7.5 Hz), 7.91 (1H, d, *J* = 8.1 Hz).

3.5.4.38. 1-Benzenesulfonyl-N-Boc-D-tryptophan (37R).

37R was synthesized according to the general procedure B: product was obtained as an off-white solid (2.18 mmol, 87%) LC-MS: tr = 3.03 min, purity 99% (at 215 nm), *m/z* 442.9 [M-H]⁻, 388.8 [M-C₄H₉+H]⁺, 345.0 [M-Boc+H]⁺. ¹H NMR (CD₃OD, 300 MHz): δ 1.38 (9H, s), 3.04 (1H, m), 3.26 (1H, m), 4.42 (1H, m), 7.21~7.32 (2H, m), 7.44~7.59 (5H, m), 7.85 (2H, d, *J* = 7.8 Hz), 7.91 (1H, d, *J* = 8.1 Hz).

3.5.4.39. (S)-3-[(1-Benzenesulfonyl)-1H-indol-3-yl]-2-(tert-butoxycarbonylamino)-N-[3-(1H-imidazol-1-yl)propyl]-propanamide (38S).

38S was synthesized according to the general procedure B: product was obtained as a white powder (1.25 mmol, 83%) LC-MS: tr = 2.87 min, 99% purity (at 215 nm), *m/z* 550.0 [M-H]⁻, 595.9 [M-H+2Na]⁻, 551.9 [M+H]⁺. HRMS *m/z* calculated for C₂₈H₃₄N₅O₅S [M + H]⁺ 552.2281, found 552,2273. ¹H NMR (CD₃OD, 300 MHz): δ 1.36 (9H, s), 1.78 (2H, m), 2.97~3.21 (4H, m), 3.83 (2H, t, *J* = 6.9 Hz), 4.35 (1H, t, *J* = 7.2 Hz), 6.94 (1H, s), 7.03 (1H, s), 7.20 (1H, t, *J* = 7.5 Hz), 7.28 (1H, t, *J* = 7.2 Hz), 7.41 (2H, m), 7.50 (1H, m), 7.57 (3H, m), 7.88 (2 H, d, *J* = 7.8 Hz), 7.95 (1H, d, *J* = 8.4 Hz). ¹³C NMR (CD₃OD, 75 MHz): δ 28.5, 28.6 (3C), 31.7, 37.2, 45.1, 56.1, 80.8, 114.6, 119.8, 120.5, 120.6, 120.8, 124.4, 125.8, 125.9, 127.8 (2C), 129.0, 130.5 (2C), 132.1, 135.1, 136.3, 138.4, 139.2, 157.5, 174.2.

3.5.4.40. *(R)*-3-[(1-Benzenesulfonyl)-1*H*-indol-3-yl]-2-(*tert*-butoxycarbonylamino)-*N*-[3-(1*H*-imidazol-1-yl)propyl]-propanamide (**38R**).

38R was synthesized according to the general procedure B: product was obtained as a white powder (1.11 mmol, 74%) LC-MS: tr = 2.88 min, 97% purity (at 215 nm), *m/z* 549.9 [M-H]⁻, 595.9 [M-H+2Na]⁻, 551.9 [M+H]⁺. HRMS *m/z* calculated for C₂₈H₃₄N₅O₅S [M + H]⁺ 552.2281, found 552,2289. ¹H NMR (CD₃OD, 300 MHz): δ 1.37 (9H, s), 1.78 (2H, m), 2.97~3.21 (4H, m), 3.84 (2H, t, *J* = 6.9 Hz), 4.34 (1H, t, *J* = 6.9 Hz), 6.95 (1H, s), 7.04 (1H, s), 7.21 (1H, t, *J* = 7.2 Hz), 7.29 (1H, t, *J* = 7.2 Hz), 7.43 (2H, m), 7.53 (1H, m), 7.56 (3H, m), 7.87 (2 H, d, *J* = 7.5 Hz), 7.95 (1H, d, *J* = 8.1 Hz). ¹³C NMR (CD₃OD, 75 MHz): δ 27.9 (3C), 28.5, 28.7, 31.7, 37.2, 45.1, 56.1, 80.7, 114.6, 119.8, 120.5, 120.8, 124.4, 125.9, 127.8 (2C), 129.0, 130.4 (2C), 132.1, 135.1, 136.3, 138.4, 139.2, 157.5, 174.2

3.5.4.41. *(S)*-2-Amino-3-[(1-benzenesulfonyl)-1*H*-indol-3-yl]-*N*-[3-(1*H*-imidazol-1-yl)propyl]-propanamide (**39S**).

39S was synthesized according to the general procedure B: product was obtained as a syrup (0.40 mmol, 60%) LC-MS: tr = 2.79 min, 100% purity (at 215 nm with basic analytical condition), *m/z* 450.6 [M-H]⁻, 496.6 [M-H+2Na]⁻, 452.5 [M+H]⁺. ¹H NMR (CD₃OD, 300 MHz): δ 1.67 (2H, m), 3.02 (2H, m), 3.24 (2H, m), 3.76 (2H, m), 4.00 (1H, m), 7.05 (1H, s), 7.08 (1H, s), 7.30 (2H, m), 7.43~7.73 (5H, m), 7.96 (3 H, m), 8.54 (1H, s).

3.5.4.42. *(R)*-2-Amino-3-[(1-benzenesulfonyl)-1*H*-indol-3-yl]-*N*-[3-(1*H*-imidazol-1-yl)propyl]-propanamide (**39R**).

39R was synthesized according to the general procedure B: product was obtained as a syrup (0.26 mmol, 63%) LC-MS: tr = 2.79 min, 100% purity (at 215 nm with basic analytical condition), *m/z* 450.5 [M-H]⁻, 496.6 [M-H+2Na]⁻, 452.5 [M+H]⁺. HRMS *m/z* calculated for C₂₃H₂₆N₅O₃S [M + H]⁺

452.1756, found 452,1764. ¹H NMR (CD₃OD, 300 MHz): δ 1.67 (2H, quint, *J* = 6.9 Hz), 3.02 (2H, t, *J* = 6.9 Hz), 3.23 (2H, dd, *J* = 4.8, 7.8 Hz), 3.77 (2H, t, *J* = 6.9 Hz), 4.01 (1H, t, *J* = 7.5 Hz), 7.05 (1H, s), 7.09 (1H, s), 7.26 (1H, t, *J* = 7.2 Hz), 7.34 (1H, t, *J* = 7.2 Hz), 7.49 (2H, t, *J* = 7.8 Hz), 7.61 (2H, m), 7.67 (1H, s), 7.74 (1H, s), 7.95 (3 H, m), 8.51 (1H, s). ¹³C NMR (CD₃OD, 75 MHz): δ 28.5, 31.5, 37.2, 45.5, 53.4, 114.7, 119.4, 120.8, 120.9, 124.5, 124.6, 125.9, 126.0, 127.9 (2C), 128.0, 128.1, 130.5 (2C), 130.6, 132.0, 135.2, 136.4, 138.2, 139.1, 163.6, 172.9.

3.5.4.43. 4-(1-Benzenesulfonyl-1H-indol-3-yl)-N-[2-(1H-imidazol-4-yl)-ethyl]-butyramide (40).

40 was synthesized according to the general procedure A and it was purified by flash column chromatography on silica gel (DCM/MeOH, 15/1) to afford the desired product as a white solid (0.57 mmol, 88%). LC-MS (10 min): tr = 4.93 min, purity > 98% (at 215 nm), *m/z* 435.0 [M-H], 481.0 [M-H+2Na], 437.0 [M+H]⁺. HRMS *m/z* calculated for C₂₃H₂₅N₄O₃S [M + H]⁺ 437.1647, found 437,1651. ¹H NMR (CD₃OD, 300 MHz): δ 1.95 (2H, quint, *J* = 7.5 Hz), 2.18 (2H, t, *J* = 7.5 Hz), 2.68 (2H, t, *J* = 7.5 Hz), 2.77 (2H, t, *J* = 7.5 Hz), 3.42 (2H, t, *J* = 7.5 Hz), 6.84 (1H, s), 7.23 (1H, t, *J* = 7.8 Hz), 7.31 (1H, t, *J* = 7.8 Hz), 7.43~7.61 (6H, m), 7.90 (2H, d, *J* = 7.5 Hz), 7.98 (1H, d, *J* = 8.4 Hz). ¹³C NMR (CD₃OD, 75 MHz): δ 25.0, 26.3, 27.7, 36.4, 40.2, 114.7, 117.9, 120.6, 124.1, 124.3, 125.7, 127.6 (2C), 130.2 (2C), 132.2, 134.9, 135.8, 136.0, 136.6, 139.0, 175.4.

3.5.4.44. 4-[1-(benzenesulfonyl)indol-3-yl]-N-(2-pyrrolidin-1-ylethyl)butanamide (41).

41 was synthesized according to the general procedure A and it was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product as a colorless oil 50 (0.45 mmol, 54%). LC-MS (10 min): tr = 5.16 min, purity > 95% (at 215 nm), *m/z* 454.0 [M+H]⁺. HRMS *m/z* calculated for C₂₆H₃₄N₃O₅S [M + H]⁺ 454.2164, found 454,2170. ¹H NMR (DMSO-*d*₆, 300 MHz): δ 1.55-1.64 (m, 2H), 1.75-1.65 (m, 4H), 1.79-1.89 (m, 2H), 2.10 (t, *J* = 7.2 Hz), 2.47-4.64 (m, 8H), 3.07 (dd, *J* = 12.4 and 6.8 Hz), 7.25 (dd, *J* = 7.8 and 1.2 Hz), 7.34 (dd, *J* = 7.8 and 1.2 Hz), 1.53-1.59 (m, 4H), 1.63-1.69 (m, 1H), 7.88-7.96 (m, 4H).

3.5.4.45. N-(3-imidazol-1-yl-propyl)-4-(2-oxo-2,3-dihydro-1H-indol-3-yl)-butyramide (42).

To a solution of indole-3-butyric acid **42a** in DMSO was added HCl 12N solution dropwise. The mixture was left to stir 30 min at room temperature. Water was added and the precipitate was collected with filter, washed with water and dried to afford the desired product **42b** as brown yellow solid (3.82 mmol, 78%). LC-MS: tr = 1.85 min, purity 87% (at 215 nm) (97% at 254 nm), *m/z* 218.0 [M-H], 219.9 [M+H]⁺, 201.9 [M-H₂O+H]⁺. To the mixture of the acid **42b**, HOBt (1.5 eq.) and EDCI (2.0 eq.) in DCM

was added DIPEA (4.0 eq.). After being stirred for 10 min at room temperature, 1-(3-aminopropyl)imidazole (1.5 eq.) was added. The mixture was stirred for 6h at room temperature. The solution was diluted with DCM, successively washed twice with aqueous saturated NaHCO₃ solution, brine, dried over MgSO₄, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (DCM/MeOH, 8/1) to afford the desired product **42** as a colorless oil (0.14 mmol, 14%). LC-MS: tr = 1.58 min, 100% purity (at 215 nm), *m/z* 326.1 [M-H]⁻, 328.0 [M+H]⁺. HRMS *m/z* calculated for C₁₈H₂₃N₄O₂ [M + H]⁺ 327.1821, found 327,1824. ¹H NMR (300 MHz, CD₃OD): δ 1.60 (2H, m), 1.98 (4H, m), 2.20 (2H, t, *J* = 7.5 Hz), 3.15 (2H, t, *J* = 6.6 Hz), 4.04 (2H, t, *J* = 6.9 Hz), 6.90 (1H, d, *J* = 7.8 Hz), 6.98 (1H, s), 7.00 (1H, t, *J* = 7.5 Hz), 7.14 (1H, s), 7.20 (1H, t, *J* = 7.5 Hz), 7.25 (1H, d, *J* = 7.5 Hz), 7.69 (1H, s). ¹³C NMR (CD₃OD, 75 MHz): δ 25.1, 31.9, 33.2, 37.3, 45.5, 51.9, 117.2, 118.1, 120.6, 129.0, 130.2, 130.4 (2C), 134.7, 135.0, 135.1, 135.1, 138.5 (2C), 142.8, 174.8.

3.5.4.46. 4-(1-benzenesulfonyl-1H-indol-2-yl)-N-(3-imidazol-1-yl-propyl)-butyramide (**43**).

Benzenesulfonyl chloride (1.05 eq.) was added dropwise into a solution of 2-iodoaniline **43a** in pyridine. The mixture was stirred overnight at room temperature. The solution was diluted with EtOAc, washed twice with 1M HCl solution and brine, dried over MgSO₄ and concentrated under reduced pressure to afford **43b** as brown solid (2.70 mmol, 90%). LC-MS: tr = 2.80 min, 96% purity (at 215 nm), *m/z* 357.8 [M-H]⁻, 379.7 [M+H]⁺. DIPEA (3.0 eq) and propylphosphonic anhydride (2.0 eq., 50 % in EtOAc) were added to a solution of 5-hexynoic acid **43c** (1.1 eq.) in EtOAc before 1-(3-aminopropyl)imidazole was added. The mixture was stirred for 4h at room temperature. The solution was diluted with EtOAc, washed with aqueous saturated NaHCO₃ solution and brine, dried over MgSO₄ and under reduced pressure to afford the crude alkyne **43d** as a sticky oil (0.75 mmol, 75%), which was directly used in next step without further purification. LCMS: tr = 1.53 min. The mixture of **43b** (188 mg, 0.52 mmol), the alkyne **43d** (1.2 eq.), CuI (0.2 eq.), Pd(PPh₃)₂Cl₂ (0.1 eq.), and Et₃N (10 eq.) in DMF was stirred at 80°C under microwave irradiation for 20min. The reaction mixture was diluted with EtOAc, washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product **43** as a colorless oil (0.12 mmol, 23%). LC-MS: tr = 2.30 min, 100% purity (at 215 nm), *m/z* 448.9 [M-H]⁻, 495.0 [M+2Na-H]⁻, 450.9 [M+H]⁺. HRMS *m/z* calculated for C₂₄H₂₇N₄O₃S [M + H]⁺ 451.1804, found 451,1805. ¹H NMR (300 MHz, CD₂Cl₂): δ 1.98 (2H, quint, *J* = 6.6 Hz), 2.11 (2H, quint, *J* = 7.4 Hz), 2.31 (2H, t, *J* = 7.4 Hz), 3.08 (2H, t, *J* = 7.5 Hz), 3.25 (2H, q, *J* = 6.3 Hz), 3.99 (2H, t, *J* = 6.8 Hz), 6.48 (1H,

s), 6.56 (1H, brs), 7.05 (2H, brs), 7.20-7.31 (2H, m), 7.39-7.57 (5H, m), 7.73 (2H, d, $J = 8.1$ Hz), 8.13 (1H, d, $J = 8.1$ Hz). ^{13}C NMR (75 MHz, CD_2Cl_2): δ 24.9, 28.5, 31.2, 35.7, 36.5, 44.7, 109.5, 114.7, 120.3, 123.7, 124.0, 126.2, 129.3, 129.9, 133.8, 137.1, 138.6, 141.5, 172.5.

3.5.4.47. 4-(1-benzenesulfonyl-1H-indazol-3-yl)-N-(3-imidazol-1-yl-propyl)-butyramide (**44**).

A mixture of indazole **44a** (0.5 g, 4.2 mmol), I_2 (2.0 eq.) and KOH (3.8 eq.) in DMF and H_2O (7/1 (v/v)) was stirred overnight at room temperature. The solution was poured into aqueous $\text{Na}_2\text{S}_2\text{O}_3$ 5% solution. The precipitate was collected by filter, washed with H_2O and dried under reduced pressure to afford the 3-iodoindazole as off-white powder (73% yield). LCMS: $t_r = 2.43$ min, m/z 242.8 [M-H] $^-$, 244.8 [M+H] $^+$. A mixture of 3-iodoindazole, Bu_4NHSO_4 (0.05 eq.) and KOH (3.0 eq.) in DCM was stirred for 10 min. Benzenesulfonyl chloride (1.1 eq.) was added and left to stir for 3h at room temperature before the reaction mixture was diluted with DCM and filtered. The filtrate was washed with brine, dried over MgSO_4 and concentrated under reduced pressure to afford the desired product **44b** as white crystal (1.49 mmol, 99%). LC-MS: $t_r = 3.10$ min, 100% purity (at 215 nm), m/z 382.8 [M-H] $^-$, 384.7 [M+H] $^+$. A mixture of Zn dust (3.0 eq.) and I_2 (3.0 eq.) in DMA under argon atmosphere was heated at 80°C for 5 min. To the mixture was added **44b** dissolved in DMA. The mixture was stirred over the week-end at 80 °C. To the mixture was added $\text{NiCl}_2(\text{PPh}_3)_2$ (0.1 eq.) and ethyl 4-bromobutyrate (3.0 eq.). The mixture was left to stir overnight at 80 °C. The solvent was removed under reduced pressure. The residue was diluted with EtOAc, washed with saturated NH_4Cl solution and brine, dried over MgSO_4 and concentrated under reduced pressure to afford the desired ethyl ester compound. ^1H NMR (300 MHz, CD_2Cl_2): δ 1.23 (3H, t, $J = 7.2$ Hz), 1.76 (2H, q, $J = 7.2$ Hz), 2.44 (2H, t, $J = 7.2$ Hz), 3.66 (2H, t, $J = 6.9$ Hz), 4.07 (2H, q, $J = 7.2$ Hz), 7.06 (1H, d, $J = 8.1$ Hz), 7.48-7.63 (4H, m), 7.66-7.72 (3H, m), 7.75 (1H, d, $J = 7.5$ Hz). ^{13}C NMR (75 MHz, CD_2Cl_2): δ 14.0, 23.6, 30.7, 50.7, 60.4, 115.9, 116.5, 127.9, 129.0, 129.2, 129.5, 133.4, 133.5, 134.1, 137.6, 141.5, 172.5. LC-MS: $t_r = 2.77$ min, m/z 370.9 [M-H] $^-$, 372.8 [M+H] $^+$, 326.9 [M-EtOH+H] $^+$. To the above ester was added NaOH (3.0 eq.), MeOH and H_2O (3/1 (v/v)). The mixture was stirred overnight at room temperature. MeOH was removed under reduced pressure before the aqueous residue was diluted with H_2O , adjusted to pH ~ 4 with HCl 1N and extracted three times with EtOAc. The combined organic layer was washed with brine, dried over MgSO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography over silica gel (cyclohexane/EtOAc, 1/1) to afford the desired product **44c** as white solid (0.36 mmol, 48% for 2 steps). ^1H NMR (300 M, CD_3OD): δ 1.69 (2H, quint, $J = 7.2$ Hz), 2.47 (2H, t, $J = 7.2$ Hz), 3.68 (2H, t,

$J = 6.6$ Hz), 7.04 (1H, d, $J = 8.1$ Hz), 7.52-7.63 (4H, m), 7.66-7.77 (3H, m), 7.81 (1H, d, $J = 7.5$ Hz). LC-MS: tr = 2.32 min, m/z 343.0 [M-H]⁻, 344.8 [M+H]⁺, 326.9 [M-H₂O+H]⁺. To a mixture of **44c**, HOBT (1.5 eq.) and EDCI (2.0 eq.) in DCM was added DIPEA (4.0 eq.). After being stirred for 10 min at room temperature, 1-(3-aminopropyl)-imidazole (1.5 eq.) was added and the mixture was stirred overnight at room temperature. It was diluted with DCM, successively washed twice with aqueous saturated NaHCO₃ solution, brine, dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (DCM/MeOH, 20/1) to afford the desired product **44** as colorless viscous oil (66% yield). LC-MS: tr = 1.97 min, 98% purity (at 215 nm), m/z 450.0 [M-H]⁻, 495.9 [M-H+2Na]⁻, 451.9 [M+H]⁺. HRMS m/z calculated for C₂₃H₂₆N₅O₃S [M + H]⁺ 452,1756, found 452,1747. ¹H NMR (300 MHz, CD₃OD): δ 1.71 (2H, quint, $J = 6.9$ Hz), 1.96 (2H, quint, $J = 6.9$ Hz), 2.39 (2H, t, $J = 7.2$ Hz), 3.16 (2H, t, $J = 6.6$ Hz), 3.67 (2H, t, $J = 6.0$ Hz), 4.05 (2H, t, $J = 7.2$ Hz), 6.96 (1H, s), 7.00 (1H, d, $J = 8.1$ Hz), 7.14 (1H, s), 7.50-7.61 (4H, m), 7.63-7.72 (4H, m), 7.81 (1H, d, $J = 7.5$ Hz).

3.5.4.48. 6-(benzenesulfonyl-phenyl-amino)-hexanoic acid (3-imidazol-1-yl-propyl)-amide (**45**).

A mixture of iodobenzene (4.1 g, 20 mmol), 6-aminocaproic acid **45a** (3.95 g, 30 mmol), K₃PO₄·H₂O (9.2 g, 40 mmol), CuI (380 mg, 2 mmol), deanol (6 ml) and H₂O (20 ml) was heated for 48 h at 80 °C under argon atmosphere (LCMS showed the reaction was quite clean). After being cooled, it was diluted with H₂O, adjusted to pH 4~5 and extracted with EtOAc (3 times). The combined organic layer was washed with brine, dried over MgSO₄, filtered and concentrated by rotary evaporator in vacuo. The residue was purified by flash column chromatography on silica gel (cyclohexane/EtOAc, 1/1) to give 6-phenylamino-hexanoic acid **45b** as pale white solid (2.40 mmol, 12% yield). LC-MS: tr = 2.22 min, m/z 206.4 [M-H]⁻, 208.5 [M+H]⁺. ¹H NMR (300 MHz, CDCl₃): δ 1.53 (2H, m), 1.75 (4H, m), 2.44 (2H, t, $J = 7.5$ Hz), 3.18 (2H, t, $J = 6.9$ Hz), 6.71 (2H, d, $J = 7.5$ Hz), 6.82 (1H, t, $J = 7.2$ Hz), 7.27 (2H, t, $J = 7.5$ Hz), 7.90 (2H, s). ¹³C NMR (75 MHz, CDCl₃): δ 24.6, 26.6, 29.1, 34.2, 44.2, 113.5, 117.9, 129.4, 148.0, 179.9. To the solution of **45b** in DCM and pyridine (3/1 (v/v)) before benzenesulfonyl chloride was added (1.02 eq.) and left to stir overnight at room temperature. It was diluted with DCM, washed twice with 1M HCl solution and brine, dried over MgSO₄, and concentrated under reduced pressure to afford the desired product **45c** as light brown solid (1.45 mmol, 90%). LC-MS: tr = 2.70 min, m/z 346.0 [M-H]⁻, 348.0 [M+H]⁺. ¹H NMR (300 MHz, CDCl₃ and CD₃OD): δ 1.39 (4H, m), 1.58 (2H, quint, $J = 7.2$ Hz), 2.28 (2H, t, $J = 7.2$ Hz), 3.54 (2H, t, $J = 6.3$ Hz), 7.00 (2H, m), 7.27 (3H, m), 7.42 (2H, m), 7.55 (3H, m), 10.1 (1H, brs). ¹³C NMR (75 MHz, CDCl₃ and CD₃OD): δ 28.2, 29.7, 31.7, 37.7, 54.3, 131.5, 132.0, 132.67,

132.74, 133.0, 136.7, 141.8, 142.6, 180.2. To the mixture of **45c** (181 mg, 0.52 mmol), HOBt (1.5 eq.) and EDCI (2.0 eq.) in DCM was added DIPEA (4.0 eq.). After stirred for 10 min at ambient temperature, 3-imidazol-1-yl-propylamine (1.5 eq.) was added and left to stir overnight at room temperature. The solution was diluted with DCM, successively washed twice with aqueous saturated NaHCO₃ solution and brine, dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (DCM/MeOH, 18/1) to afford the desired product **45** as a colorless oil (0.40 mmol, 76%). LC-MS: tr = 2.12 min, > 94% purity (at 215 nm), *m/z* 453.1 [M-H]⁻, 499.1[M-H+2Na]⁻, 455.9 [M+H]⁺. HRMS *m/z* calculated for C₂₄H₃₁N₄O₃S [M + H]⁺ 455,2117, found 455,2107. ¹H NMR (300 MHz, CD₃OD): δ 1.41 (4H, m), 1.56 (2H, quint, *J* = 6.9 Hz), 1.99 (2H, quint, *J* = 6.9 Hz), 2.17 (2H, t, *J* = 7.2 Hz), 3.18 (2H, t, *J* = 6.9 Hz), 3.62 (2H, m), 4.06 (2H, t, *J* = 6.9 Hz), 6.98 (1H, s), 7.03 (2H, m), 7.17 (1H, s), 7.31 (3H, m), 7.48-7.60 (4H, m), 7.65 (1H, m), 7.69 (1H, s). ¹³C NMR (75 MHz, CD₃OD): δ 25.0, 25.6, 27.5, 30.6, 35.5, 36.0, 44.2, 50.0, 119.3, 127.3, 127.7, 128.6, 128.7, 132.6, 137.1, 138.0, 138.7, 174.8.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Pierre Boulens and Marion Gauriot for technical assistance and Pr W.J. Tang for recombinant IDE. The authors acknowledge financial support from INSERM, University of Lille, Institut Pasteur de Lille, Region Hauts-de-France, l'Agence Nationale de la Recherche ANR grants JCJC11-JS07-015-01, « European Genomic Institute for Diabetes » E.G.I.D, ANR-10-LABX-0046, under the frame program Investissements d'Avenir I-SITE ULNE / ANR-16-IDEX-0004 ULNE, INSERM PCSI grant (N°ASC20017ESA), Fondation pour la Recherche Médicale FRM grant (N°DCM20111223046), State (0823007, 0823008, 07-CPER 009-01, 2007-0172-02-CPER/3), the European Union under the European Regional Fund (ERDF), by the Hauts-de-France Regional Council (N°17003781), the MEL (N°2016_ESR_05), and the French State (N°2017-R3-CTRL-Phase 1). The NMR facilities were funded by the Region Hauts-de-France, CNRS, Institut Pasteur de Lille, European Regional Fund (ERDF), Ministère de l'Enseignement supérieur, de la Recherche et de l'Innovation (MESRI) and Lille University. N. K. is a recipient of a PhD fellowship from the University of Lille.

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Graphical Abstract

