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# Introduction of the 4-(4-bromophenyl)benzenesulfonyl group to hydrazide analogs of Ilomastat leads to potent gelatinase B (MMP-9) inhibitors with improved selectivity

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ABSTRACT

Hydrazide derivatives of Ilomastat, carrying either aryl groups or distinct alkyl and arylsulfonyl moieties were synthesized and evaluated for their MMP inhibitory activity. Potent and selective MMP-9 inhibition ($IC_{50}$ = 3 nM) was observed for compound **3m** (arylsulfonyl group: 4-(4-Br–$C_6H_4$)–$C_6H_4$–$SO_2$–). Interaction with the $S_2$ enzyme subsite is mainly responsible for the inhibitory properties of this derivative as confirmed by molecular docking computation.



## 1. Introduction

Matrix metalloproteinases (MMPs) belong to the $M_{10}$ clan of zinc containing endopeptidases that have been shown to play an important role in a variety of physiological functions.[1] Their activity is controlled by endogenous inhibitors, mainly tissue inhibitors of metalloproteinases (TIMP). Disruption of the finely tuned MMPs/TIMPs balance is observed in several pathologies including osteoarthritis, atherosclerosis, aneurysm, pulmonary emphysema, neurodegenerative diseases, and cancer.

Consequently, the development of synthetic MMP inhibitors (MMPIs) possessing a high potency and selectivity has attracted significant efforts aiming to interfere with the progression of these pathologies.[2]

To date a myriad of broad spectrum MMP inhibitors has been synthesized but the results of the first clinical trials, notably in cancer area, proved to be rather disappointing due to the multifaceted functions of this endopeptidase family.[3]

Indeed, besides acting as matrix degrading enzymes, MMPs can hydrolyze or activate cytokines and growth factors, shed or activate receptors, and modulate the control of proteinases from other families through proteolysis of proteinase inhibitors. Important side effects and lack of selectivity owing to similarities of the active sites of other metalloproteinases (ACE, ADAM, ADAMT) were also reported to be responsible for the inefficiency of early MMPIs.[4] Finally, clinical trials have also evidenced that some MMPIs may have disease-promoting or suppressing activities, leading to the recent classification of MMPs as being either target or antitarget in cancer progression.[5] It needs to be emphasized that gelatinases, that is, MMP-2 and MMP-9 have been considered to play a pivotal role in the development of several cancer types and aneurysm formation.

A vast majority of the thousands competitive MMPIs bears an hydroxamic acid moiety as zinc-binding group (ZBG).[6] However, hydroxamate-type inhibitors display several drawbacks as low biodisponibility and stability as well as poor pharmacokinetic properties. In addition, the high potency of hydroxamic acid as zinc chelator might be somewhat disadvantageous since it can mask the contribution of other subsites in enzyme inhibition. This lack

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of selectivity has triggered important synthetic efforts to develop alternative ZBGs such as carboxylate, thiol, phosphonic acid, reversed hydroxamate, sulfonamide functions and other heterocycles[7] and to better exploit the neighboring subsites ($S_1'$, $S_2'$..., $S_1$, $S_2$...).

To that respect, we recently initiated a research program supported by molecular modeling to identify MMPIs containing novel zinc-binding groups.[8] For our pharmacomodulation we chose Ilomastat **1**, a potent but non-selective hydroxamic acid containing inhibitor (Fig. 1).[9] From this preliminary investigation a few hydrazide-type functions (**2**) have emerged as novel ZBGs. A second series of Ilomastat analogs (**3**) bearing sulfonylhydrazide-type ZBG has also been proposed. By incorporating a more electron-withdrawing arylsulfonyl group on the hydrazide function we envisaged to possibly reinforce the interactions between our inhibitors and the Glu residue in MMP active site (Fig. 2).[10]

Importantly, hydrazide-type ZBG presents the advantage to extend the molecule to the left-hand side thus allowing to study the contribution of non-prime MMP subsite ($S_1$, $S_2$) occupancy in inhibition potency and selectivity. Such bis-arylsulfonylamide-type group containing inhibitors have already been described[11] occupying mainly the $S_1'$ enzyme subsite.

As an extension of our program, herein we report in full details the synthesis and biological evaluation of new hydrazide (**2**) and sulfonylhydrazide (**3**) group containing Ilomastat analogs. In addition, a complementary study has been carried out from the most potent 4-(4-bromophenyl)benzenesulfonylhydrazide derivative to better exploit $S_1'$ and $S_2'$ enzyme subsites. To this end, different alkyl(aryl)alkylidene side chains and 2-phenyl substituted tryptophanamide were incorporated into the pseudopeptide backbone (analogs of type **4**). Inhibitory activities were measured on the main MMPs and the results were analyzed by molecular docking experiments.

## 2. Chemistry

### 2.1. Synthesis of Ilomastat analogs containing hydrazide (2) or sulfonylhydrazide functions (3) as ZBG

The synthesis of the derivatives containing hydrazide- or sulfonylhydrazide functions as ZBG followed a linear pathway (Scheme 1). Pseudopeptide backbone **5** was assembled by 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-methylmorpholinium chloride (DMTMM) assisted coupling[12] between *N*-methyltryptophanamide **6** and functionalized chiral succinic ester-acid **7**[13] (Scheme 1).

For the introduction of new ZBGs, two pathways were elaborated. The first one, consisted in the direct amidification of the carboxylic acid function of **5** with arylhydrazines. In this way, arylhydrazides **2a–d** were obtained in moderate (45–52%) yield after purification by chromatography. Coupling of acid **5** with aryl(or methyl)sulfonylhydrazines according to the same pathway afforded the corresponding sulfonylhydrazides **3a–d** in moderate yield. Sulfonylhydrazines used for direct coupling were prepared by sulfonylation of *N*-Boc-hydrazine followed by hydrochloric acid mediated deprotection.[14] We attributed the low yield of the direct coupling to the low nucleophilic character of sulfonylhydrazines.

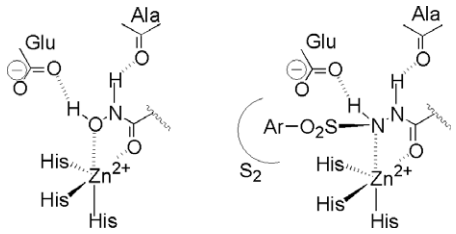



**Figure 2.** Bindings of hydroxamate and (sulfonyl)-hydrazide type ZBG.

To overcome such drawbacks, we tried a two-step pathway via sulfonylation of the corresponding non-substituted hydrazide **2f** which was prepared by coupling **5** with *N*-Boc-hydrazine and subsequent acid catalyzed deprotection of **2e**. Sulfonylation of **2f** afforded the targeted arylsulfonylhydrazide Ilomastat analogs **3e–m** in variable yields (11–70%). 2-Aminophenyl substituted analog **3k** was obtained by catalytic reduction (10% Pd/C, $H_2$) of the corresponding nitro derivative **3f**. Aliphatic long chain analogs **3g,h** were prepared using dodecansulfonylchloride and hexadecansulfonychloride as sulfonating agent, respectively.

### 2.2. Synthesis of alkyl(aryl)alkylidene side-chain bearing Ilomastat analogs with 4-(4-bromophenyl)phenylsulfonylhydrazide as zinc-binding group (4)

The synthesis of these Ilomastat analogs was based on a similar linear approach starting from monoesters **7** or **9**[15,16] and (*S*)-tryptophanmethylamide (**6**)[17] or its 2-phenyl-subtituted counterpart (**8**).[18] DMTMM-assisted coupling of **6** or **8** with (*E*)-monoallyl esters **9v**,**w** or with chiral ester-acid **7** smoothly afforded the corresponding succinyltryptophanamide derivatives **10v–y** or **11z**, respectively. Deprotection of esters **10v–y** ($Pd(PPh_3)_4$) or **11z** (TFA) led to the free acids **12v–z**.

The final products **4v,w,x,z** were obtained by coupling the free acids **12v,w,x,z** with 4-(4-bromophenyl)benzenesulfonylhydrazide hydrochloride. Product **4y** was prepared in three steps from **12y** via the Boc-hydrazide analog **13y**. Deprotection of this latter gave **14y** which was sulfonylated with 4-(4-bromophenyl)benzenesulfonylchloride to afford the aryl-alkylidene side-chain containing Ilomastat analog **4y** (Scheme 2).

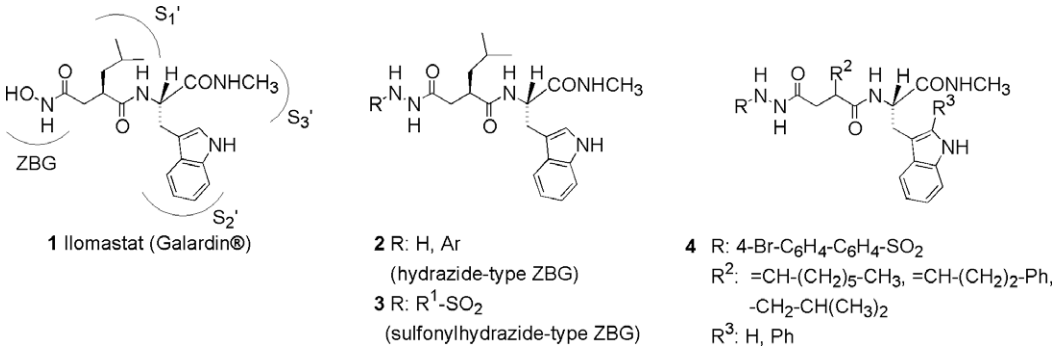
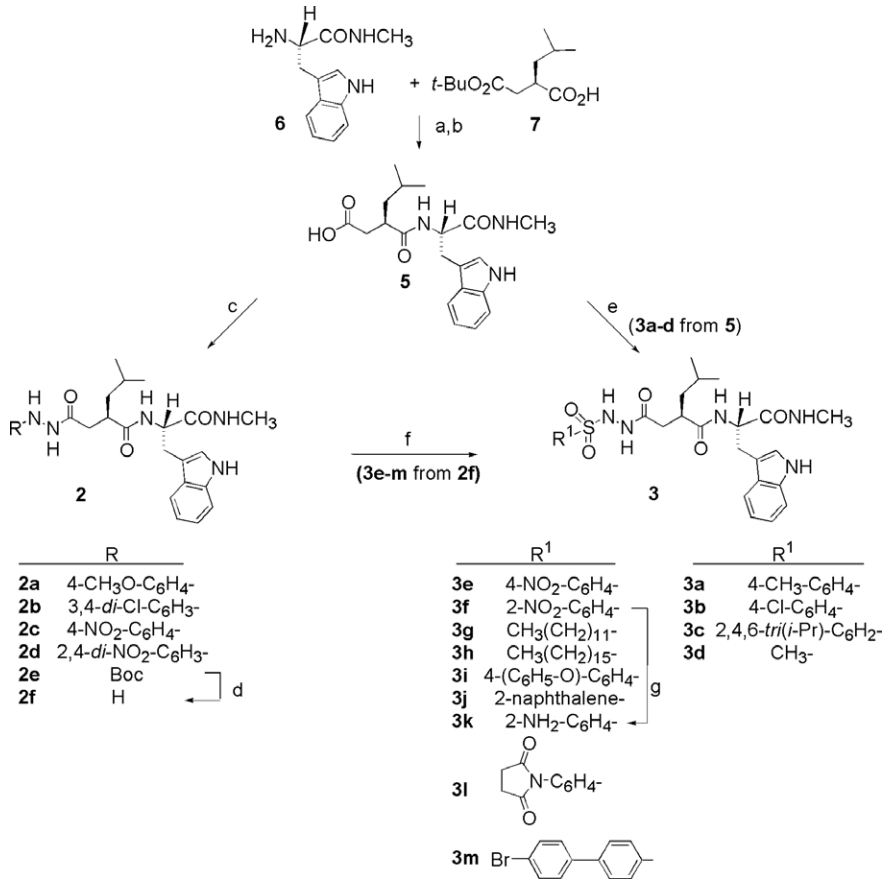
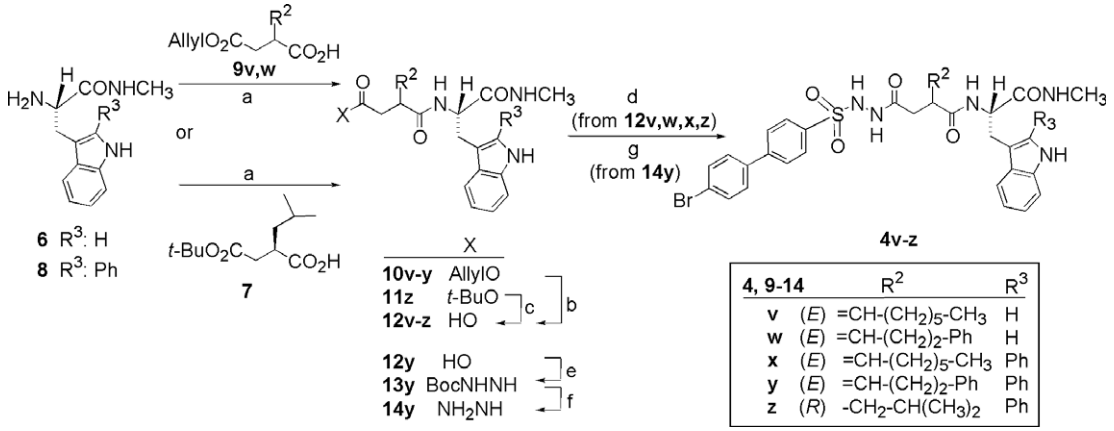



**Figure 1.** Ilomastat (**1**), its hydrazide (**2**), sulfonylhydrazide (**3**), and various alkylidene side-chain containing analogs (**4**).




**Scheme 1.** Synthesis of hydrazide (**2**) and sulfonylhydrazide analogs (**3**) of Ilomastat. Reagents and conditions: (a) DMTMM, MeOH, 69–81%; (b) TFA, $CH_2Cl_2$, 57–80%; (c) $R^1NHNH_2$ (or HCl salt), DMTMM, NMM, THF, 45–92%; (d) EtOH/HCl, 73%; (e) $R^2SO_2NHNH_2$ (or HCl salt), DMTMM, NMM, THF, 11–67%; (f) $R^2SO_2Cl$, pyridine (or $Et_3N$, or $NaHCO_3$), THF (co-solvent DMSO), 0 °C, 11–70%; (g) Pd–C, $H_2$, EtOH, 57%.




**Scheme 2.** Synthesis of alkyl(aryl)alkylidene side-chain bearing Ilomastat analogs with 4-(4-bromophenyl)phenylsulfonylhydrazide as ZBG. Reagents and conditions: (a) DMTMM, THF, 53–93%; (b) $Pd(PPh_3)_4$, morpholine, THF, 40–74%; (c) TFA, $CH_2Cl_2$, 64%; (d) 4-Br–$C_6H_4$–$C_6H_4$–$SO_2NHNH_3^+Cl^-$, DMTMM, NMM, THF, 14–41%; (e) $BocNHNH_2$, DMTMM, NMM, THF, 92%; (f) HCl, MeOH, 55%; (g) 4-Br–$C_6H_4$–$C_6H_4$–$SO_2Cl$, $Et_3N$, THF, 54%.

## 3. Biological evaluation and molecular modeling studies

### 3.1. Enzyme inhibition experiments

Hydrazide (**2a–d**) and sulfonylhydrazide analogs (**3a–m** and **4v–z**) were tested for their ability to inhibit MMP-1 (collagenase), MMP-2 (gelatinase A), and MMP-14 (membrane type-1 matrix metalloproteinase) using the corresponding fluorimetric substrates of these enzymes. For comparison, Ilomastat (**1**) was also included in all series of biological evaluations.[13a,19] For the most active molecules, the test panel was enlarged to MMP-3 (stromelysin), MMP-7 (matrilysin), and MMP-9 (gelatinase B).

Aryl-substituted hydrazide analogs (**2a–d**) displayed low inhibitory capacity toward MMPs, whatever electron-donating or electron-withdrawing group bearing aryl-substituted hydrazides was considered (Table 1).

Among the sulfonylhydrazide analogs, the *p*-tosyl derivative **3a** was first subjected to biological evaluation toward MMP-1, MMP-

**Table 1**
Inhibition of Ilomastat (**1**) and some hydrazide (**2**) and sulfonylhydrazide (**3**) ZBG containing analogs ($IC_{50}$, nM)

| Compound | R | MMP-1 | MMP-2 | MMP-14 |
|---|---|---|---|---|
| **1** | | 0.4 | 0.4 | 5.2 |
| **2a** | 4-$CH_3O$–$C_6H_4$– | 330 | 10,000 | >10,000 |
| **2b** | 3,4-Di-Cl–$C_6H_3$– | 5600 | 10,000 | 1000 |
| **2c** | 4-$NO_2$–$C_6H_4$– | >10,000 | 10,000 | >10,000 |
| **2d** | 2,4-Di-$NO_2$–$C_6H_3$– | >10,000 | 10,000 | >10,000 |
| | $R^1$ | | | |
| **3a** | 4-Me–$C_6H_4$– | 250 | 24 | 2600 |
| **3b** | 4-Cl–$C_6H_4$– | 290 | 18 | 190 |
| **3c** | 2,4,6-Tri(*i*-Pr)–$C_6H_2$– | 4000 | 100 | 3100 |
| **3d** | $CH_3$– | 5600 | 740 | 3700 |
| **3e** | 4-$NO_2$–$C_6H_4$– | 490 | 310 | 1500 |
| **3f** | 2-$NO_2$–$C_6H_4$– | 850 | 320 | 1100 |
| **3g** | $CH_3(CH_2)_{11}$– | 980 | 1400 | 9700 |
| **3h** | $CH_3(CH_2)_{15}$– | 440 | 300 | 5100 |
| **3k** | 2-$NH_2$–$C_6H_4$– | 93 | 92 | 730 |

2, and MMP-14. Its MMP-2 enzyme inhibition ($IC_{50}$ = 24 nM) was in good correlation with formerly obtained data ($IC_{50}$ = 30 nM).[20] However, a lower MMP-2/MMP-1 selectivity (10-fold) was found. Interestingly this compound, contrary to Ilomastat, is a poor MMP-14 inhibitor.

Comparing hydrazide (**2c**) and sulfonylhydrazide (**3e**) ZBG containing analogs, the observed difference of inhibition highlighted the importance of the acid character of sulfonamide hydrogen in metal chelation. Replacement of the 4-methyl group by chlorine (**3a** vs **3b**) allowed to maintain MMP-2 and MMP-1 inhibitory activity at similar $IC_{50}$ values but selectivity toward MMP-14 was partly lost. Other sulfonamides with electron-withdrawing groups (**3e**,**f**,**k**) showed neither higher inhibition, nor better selectivity. Incorporation of tri-*iso*-propylphenyl substitution (**3c**) afforded a more selective MMP-2 vs MMP-1 profile.

$S'_1$ subsite of MMP-2 has been reported to accommodate long alkyl chain and fibronectin type II domains of both gelatinases A and B have been shown to interact with long chain fatty acids.[21] Thus, we explored the possibility that $S_2$ subsite of enzyme could similarly exhibit hydrophobic interactions. To this end, several alkylsulfonylhydrazide derivatives (**3d**,**g**,**h**) were synthesized and tested. Unfortunately, these derivatives did not display good inhibitory properties (Table 1).

On the contrary, bis-arylsulfonyl group substituted analogs displayed enhanced MMP-2 and MMP-1 enzyme inhibitory capacities (Table 2). Thus, biphenyl-ether (**3i**) or succinimidylphenyl (**3l**) substituted analogs presented good MMP-2 and MMP-9 inhibitory activities, but without real selectivity comparing to other MMPs. None of these compounds had any major influence on MMP-14 activity.

Naphthyl substitution (**3j**) enhanced matrilysin (MMP-7) and MMP-14 selectivity which was maintained by the incorporation of 4-(4-bromophenyl)phenyl group (**3m**). Among the sulfonylhydrazide analogs, the 4-(4-bromophenyl)benzene substituted one (**3m**) appeared to be the most potent gelatinase inhibitor with $K_i$ values of 8.5 and 3.6 nM for gelatinases A and B, respectively, and only weak inhibition was measured on MMP-3 (415 nM), MMP-7 (523 nM), and MMP-14 (1900 nM) (Tables 2 and 3).

As **3m** proved to be a potent and selective gelatinase inhibitor, in a second phase of our program, we were interested in studying the influence of $S'_1$ subsite substitution. Formerly, we evidenced that replacement of the *iso*-butyl group of Ilomastat by (*E*)-alkylalkylidene side-chains afforded good MMP-2 selectivity,[15] while the (*E*)-phenyl-propylidene analog showed significant MMP-9 selectivity.[16] In addition, introduction of a phenyl group in position 2 of indole of Ilomastat increases the MMP-2 inhibition ($IC_{50}$ = 0.092 nM).[22]

Combining these diverse structural modifications, we prepared a series of Ilomastat analogs (**4v**–**z**) with 4-(4-bromophenyl)benzenesulfonylhydrazide as zinc-binding group focusing on the modifications of $S'_1$ and $S'_2$ sites of the pseudopeptide backbone (Table 4).

Generally, 4-(4-bromophenyl)benzenesulfonylhydrazide type analogs (**4v**–**z**) were less potent inhibitors than their hydroxamic acid containing counterparts. Thus, **4v** was nearly 14 times less active than corresponding hydroxamic acid analog ($IC_{50}$ = 123 nM)[16] and the MMP-2 selectivity over other MMPs was lost. Similarly weaker inhibitory activity and no effective selectivity were observed for phenylpropylidene substituted analog (**4w**).

Introduction of a phenyl group on C-2 of the indole ring in the heptylidene series (**4x** vs **4v**) had low influence on enzyme inhibition contrary to the phenylpropylidene side-chain bearing analog (**4y**) where better MMP-2 inhibition ($IC_{50}$ = 65 nM) with slight selectivity was observed. Comparing enzyme inhibitions of **4z** with **3m**, it is interesting to note that 2-phenyltryptophan containing

**Table 3**
Relative selectivity data of some substituted bis-arylsulfonylhydrazide ZBG containing derivatives (**3j**,**l**,**m**)

| Compound | Selectivity (vs MMP-9) $R^1$ | MMP-1/ MMP-9 | MMP-2/ MMP-9 | MMP-3/ MMP-9 | MMP-7/ MMP-9 | MMP-14/ MMP-9 |
|---|---|---|---|---|---|---|
| **1** | | 0.7 | 0.7 | 43 | — | 8.5 |
| **3j** | 2-Naphthalene- | 13 | 3 | 127 | 559 | 909 |
| **3l** | 4-Succinylimidyl-$C_6H_4$– | 11 | 9 | 108 | 13 | 85 |
| **3m** | 4-(4-Br–$C_6H_4$)–$C_6H_4$– | 10 | 3.3 | 567 | 158 | 5666 |

**Table 4**
Inhibition of some alkyl(aryl)alkylidene side-chain containing Ilomastat analogs with 4-(4-bromophenyl)phenylsulfonylhydrazide as zinc-binding group (**4**) ($IC_{50}$, nM)

| Compound | $R^2$ | $R^3$ | MMP-1 | MMP-2 | MMP-3 | MMP-9 | MMP-14 |
|---|---|---|---|---|---|---|---|
| **4v** | =CH–$(CH_2)_5$Me | H | NI | 1813 | 2538 | 2563 | NI |
| **4w** | =CH–$(CH_2)_2$Ph | H | 1033 | 310 | 3863 | 1246 | 105 |
| **4x** | =CH–$(CH_2)_5$Me | Ph | 2043 | 1273 | 174 | 732 | 3784 |
| **4y** | =CH–$(CH_2)_2$Ph | Ph | 1075 | 65 | 463 | 827 | NI |
| **4z** | –$CH_2$–$CH(CH_3)_2$ | Ph | 350 | 247 | 53 | 18 | 1237 |

NI, no inhibition.

**Table 2**
Inhibition of some substituted aryl- or bis-arylsulfonylhydrazide ZBG containing derivatives (**3i**,**j**,**l**,**m**) ($IC_{50}$, nM)

| Comound | $R^1$ | MMP-1 | MMP-2 | MMP-3 | MMP-7 | MMP-9 | MMP-14 |
|---|---|---|---|---|---|---|---|
| **1** | | 0.4 | 0.4 | 26 | — | 0.6 | 5.2 |
| **3i** | 4-($C_6H_5$–O)$C_6H_4$– | 110 | 45 | — | — | — | 2800 |
| **3j** | 2-Naphthalene- | 150 | 37 | 1400 | 6150 | 11 | 10,000 |
| **3l** | 4-Succinylimidyl-$C_6H_4$– | 91 | 79 | 920 | 110 | 8.5 | 720 |
| **3m** | 4-(4-Br–$C_6H_4$)–$C_6H_4$– | 30 | 9.8 | 1700 | 475 | 3 | 17,000 |
| **3m** | 4-(4-Br–$C_6H_4$)–$C_6H_4$– | | $K_i$ = 8.5 | $K_i$ = 415 | $K_i$ = 523 | $K_i$ = 3.6 | $K_i$ = 1900 |

—, not tested.

analog **4z** conserved important gelatinase inhibitory ability (MMP-2 and MMP-9: $IC_{50}$ = 247 and 18 nM, respectively) associated to a potent MMP-3 inhibitory activity ($IC_{50}$ = 53 nM).

### 3.2. Molecular modeling studies

The docking computations by Autodock[23,24] have been carried out with several hydrazide compounds using MMP-2 and MMP-9 as target proteins. The structural analysis of the lowest energy model for each MMP will be detailed. The residue numberings employed for each MMP are those used in the Swissprot database.[25]

In keeping with enzyme inhibition studies, we initially focused our efforts on the 4-(4-bromophenyl)benzenesulfonylhydrazide zinc-binding group containing Ilomastat analog **3m** which potently and selectively inhibited gelatinases (MMP-2 and MMP-9).

The lowest energy model proposed for compound **3m** showed a similar docked position at the surface of both MMP-2 and MMP-9 (Figs. 3 and 4). As expected, the 4-(4-bromophenyl)phenyl group of **3m** is localized in the $S_2$ subsite of MMP-2 and presents van der Waals interactions with residues $Asn_{111}$, $Gly_{181}$-Tyr-Pro-$Phe_{184}$, $Leu_{190}$, and $Ala_{192}$-His-$Ala_{194}$. According to our previous reported model[20] the sulfonylhydrazide group occupies the $S_1$ subsite and chelates the zinc atom. The sulfonylhydrazide group forms further two H-bonds with backbone of residues $Ala_{192}$ and $Ala_{194}$. The *iso*-butyl group presents van der Waals interactions with the amino acids located at the entrance of $S_1'$ subsite: $Leu_{191}$, $Ala_{192}$, $Val_{400}$, $His_{403}$, and $Ala_{404}$. The indole group is located in the $S_2'$ subsite forming van der Waals contacts with $His_{413}$, $Pro_{423}$, and $Ile_{424}$, belonging to the lower rim of the catalytic site. The pseudopeptide backbone of **3m** establishes two H-bonds with the amino acids of the lower rim ($Pro_{423}$ and $Tyr_{425}$) and two other H-bonds with $Gly_{189}$ and $Leu_{191}$ (upper rim).

The position and the orientation of the compound **3m** docked to MMP-2 and MMP-9 are similar as confirmed by the root mean square deviation (RMSD) value of 0.91 Å, calculated between the atoms of the two inhibitor models. At the MMP-9 surface, the 4-(4-bromophenyl)phenyl group has van der Waals contacts with $Phe_{110}$, $Tyr_{179}$, $Pro_{180}$, $Leu_{187}$, and $Ala_{189}$-His-$Ala_{191}$ residues (MMP-9 numbering). The sulfonylhydrazide group is engaged in two H-bonds with the amino acids $Ala_{189}$ and $Ala_{191}$, respectively. The *iso*-butyl group side chain presents van der Waals interactions with $Leu_{188}$, $Ala_{189}$, $Val_{398}$, $Glu_{402}$, and $Tyr_{423}$, forming the entrance of the $S_1'$ subsite. The four H-bonds, already detected in the lowest energy model with MMP-2 as target protein, involve the residues $Gly_{186}$ and $Leu_{188}$ in the upper rim, and the amino acids $Pro_{421}$ and $Tyr_{423}$ in the lower rim of the catalytic site. The indole group, located in the $S_2'$ subsite, interacts with $Pro_{421}$ and $Met_{422}$ of MMP-9 via van der Waals forces. The measured affinity of **3m** is better for MMP-9 than for MMP-2. In our docking models, the main difference relies in the van der Waals interaction of the 4-(4-bromophenyl)phenyl group with $Phe_{110}$ of MMP-9 contrary to the interaction with $Asn_{111}$ in MMP-2. In our experimental conditions, it is most likely that a π-stacking interaction occurs between the 4-(4-bromophenyl)phenyl group of **3m** and $Phe_{110}$ of MMP-9, thus explaining the observed affinity differences (Table 2). Moreover, the $S_2$ pocket in MMP-9 is smaller than in MMP-2. Accordingly, the 4-(4-bromophenyl)phenyl group can easily insert into the $S_2$ pocket of both gelatinases, but the smaller size of MMP-9 induces stronger van der Waals interactions favoring its binding.

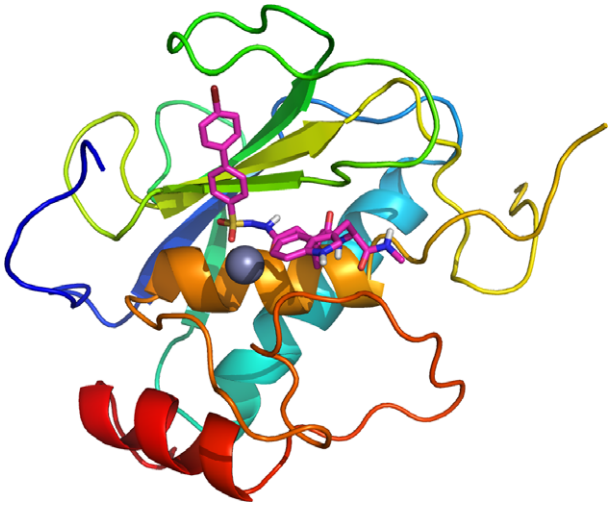


**Figure 3.** Lowest energy model for the interactions between **3m** and the catalytic site of MMP-2. (C atoms are colored in magenta, the MMP-2 is in cartoon representation; and the zinc atom is displayed in grey sphere.)

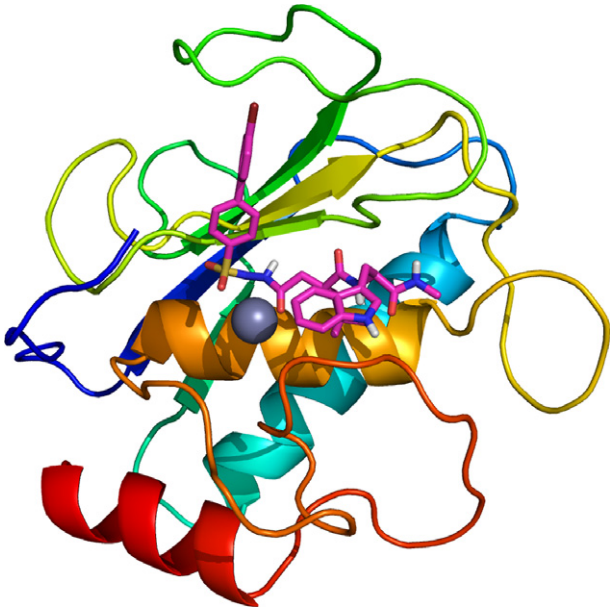


**Figure 4.** Lowest energy model for the interactions between **3m** and the catalytic site of MMP-9. (C atoms are colored in magenta, the MMP-9 is in cartoon representation; and the zinc atom is displayed in grey sphere.)

Introduction of a phenyl group at $R^3$ afforded a less potent inhibitor of gelatinases, particularly MMP-2 inhibitor (**4z** vs **3m**). Accordingly, we attempted to explain differences in MMP-9 inhibition of **4z** as compared to **3m** ($IC_{50}$ = 18 nM and 3 nM, respectively). The lowest energy model of **4z** and **3m** displayed almost similar position and conformation within the MMP-9 catalytic site (Fig. 4). The same interactions are highlighted except for the 2-phenylindole group. This group interacts with $Pro_{421}$ and $Met_{422}$, belonging to the lower rim of the MMP-9 active site, as it was observed in the lowest energy model of the compound **3m**. Moreover, phenyl ring of this group has specific van der Waals interactions with $Leu_{187}$ and $His_{411}$ (Fig. 5).

On the contrary, the molecular docking computations have failed to demonstrate any realistic model concerning the interaction between **4z** and MMP-2. In keeping with the relatively high measured $IC_{50}$ values, docking results suggested low MMP-2 affinity for **4z**. Indeed, incorporation of a phenyl group in **3m** (leading to **4z**) did not allow to obtain a coherent interaction model by docking computations with MMP-9. To explain these discrepancies, the lowest energy model of the **4z**/MMP-9 complex was superimposed with the MMP-2 structure. The superimposed structures of the MMPs are very close and **4z** fits well the catalytic site of the MMP-2, except for the 2-phenylindole group in the $S_2'$ subsite that

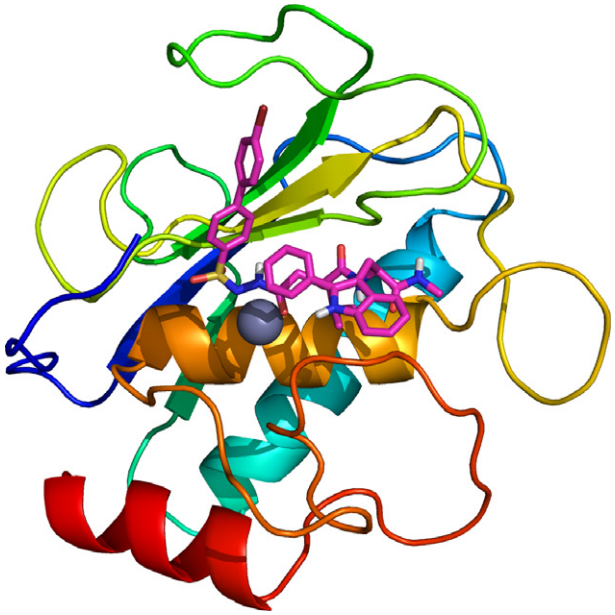


**Figure 5.** Lowest energy model for the interactions between **4z** and the catalytic site of MMP-9. (C atoms are colored in magenta, the MMP-2 is in cartoon representation; and the zinc atom is displayed in grey sphere.)

exhibits steric hindrance with $Ile_{424}$ localized in the lower rim of the MMP-2 active site. Precisely, the ethyl group of the $Ile_{424}$ side-chain adopts a particular conformation which leads to unfavorable van der Waals-type interaction. The same three-dimensional conformation of $Ile_{424}$ has also been observed in other MMPs (PDB codes: 1HOV, 1QIB, 1GXD, and 1EAK). Conversely, the equivalent amino acid in MMP-9, $Met_{422}$, has its side-chain directed in opposite direction allowing the binding of **4z** with the 2-phenylindole group in its $S_2'$ subsite. The 2-phenylindole group presents both rigid and voluminous properties and consequently cannot fit properly the $S_2'$ cleft of MMP-2. Therefore, the lower affinity of the compound **4z** for the MMP-2 is likely due to the $Ile_{424}$ side-chain localized in the $S_2'$ subsite.

Molecular docking computations for compounds **4x** and **4y** have failed to deliver realistic positions and plausible conformations for these ligands in the active site of both MMP-2 and MMP-9, suggesting a low affinity between these inhibitors and both enzymes. Therefore, a virtual derivative with a methyl group as side-chain function was built up and tested using the same protocol. Again, no correct conformation of this compound in the enzyme active site could be evidenced, indicating that its low affinity was not due to the bulkiness of the phenyl group or the alkyl chain.

In order to explain why replacement of the hydroxamate moiety by 4-(4-bromophenyl)phenylsulfonylhydrazide as zinc-binding group led to loss of enzyme inhibition selectivity we calculated the most probable conformation of the zinc chelated forms of **4x** and **4y** and docked them into the active site of MMP-2 and MMP-9. Our data indicated that both **4x** and **4y** adopted a new conformation in which $sp^2$-hybridized carbon atom of the alkylidene side-chain led to steric hindrance impeding the entrance in $S_1'$ subsite. This is particularly true for $Pro_{423}$ in MMP-2 or $Pro_{421}$ in MMP-9. Consequently, although the $S_2$ subsite is occupied no synergistic effect could be obtained as a consequence of inappropriate occupancy of primed subsites.

Finally, we tried to explain why sulfonylhydrazide ZBG containing inhibitors had good but generally lower enzyme inhibitory activity than their hydroxamic acid type analogs. Our calculations pointed out that in the case of sulfonylhydrazide structures the side-chain carrying carbon atom is displaced by 1.5–3 Å toward outside of the $S_1'$ subsite. If this crucial carbon atom is $sp^3$-hybridized, its conformational mobility allows the insertion of the side-chain in the $S_1'$ subsite affording higher inhibition activity (**3m** and **4z**).

## 4. Conclusion

From our previous investigation, we initially hypothesized that incorporation of an electron-withdrawing moiety to hydrazide analog of Ilomastat would amplify their MMP inhibition activity. Indeed, all synthesized sulfonylhydrazide derivatives appeared more active than the aryl-substituted analogs, confirming the importance of sulfonamide hydrogen in metal chelation. Increasing the electron-withdrawing character of this group did not improve their MMP inhibitory potential but elongation with diaryl moiety afforded potent derivatives that inhibit preferentially gelatinases. Strikingly, the 4-(4-bromophenyl)phenylsulfonylhydrazide analog (**3m**) inhibited MMP-9 with an $IC_{50}$ comparable to that of Ilomastat ($IC_{50}$ = 3 nM and 0.6 nM, respectively). Furthermore, this derivative possesses MMP-9 selectivity over MMP-1, MMP-3, and MMP-14 which are 14-, 13-, and 666-fold greater, respectively, than Ilomastat.

The importance of $S_3$ and $S_2$ subsites has been highlighted previously for selective inhibition.[26] Computation methods followed by statistical analysis have provided a ranking of the relative importance of the MMP subsites for inhibitor selectivity.[27] According to this study, the $S_2$ subsite is the second most important subsite for selectivity.

The 4-(4-bromophenyl)phenyl group might occupy the $S_2$ subsite of MMP-9, a contention which has been supported by molecular docking studies. The small size, as well as the presence of $Phe_{110}$ in the $S_2$ pocket of MMP-9 confer to this enzyme a better affinity for the 4-(4-bromophenyl)phenyl moiety compared to MMP-2 and probably to other MMPs.

Attempts to further improve the affinity or selectivity of **3m** through pharmacomodulation of $R^2$ or $R^3$ positions of the pseudopeptidic backbone targeting the primed subsites ($S_1'$, $S_2'$) proved to be unsuccessful mainly due to steric hindrance. Nevertheless, we have evidenced the contribution of the $S_2$ subsite in the development of MMP-9 selective inhibitors and this concept might be valuable in the design of therapeutic agents in elastic tissue disease as aneurysms.

## 5. Experimental

All solvents were of reagent grade and, when necessary, were purified and dried by standard methods. Reaction and products were routinely monitored by thin-layer chromatography (TLC) on silica gel (Kieselgel 60 $PF_{254}$, Merck). Flash chromatography was performed on Kieselgel 60 (40–63 μm, Merck). Melting points were determined on a Reichert Thermovar hot-stage apparatus and are uncorrected. UV spectra were recorded in methanol solution on a Unicam 8700 UV/vis apparatus. IR spectra were measured on a Perkin-Elmer Spectrum BX FTIR instrument. $^1H$ NMR (300 MHz) and $^{13}C$ NMR (75 MHz) spectra were recorded on a Bruker AC 300 spectrometer using TMS as internal standard, chemical shifts were given in ppm ($\delta$). Couplings expressed as s, br s, d, t, and m correspond to singlet, broad-singlet, doublet, triplet, and multiplet, respectively. Mass spectra were recorded on a MSQ ThermoFinnigan apparatus using electrospray ionization (ESI) method or on a GCT Micromass apparatus using electronimpact (EI) or chemical ionization (CI) method. Optical rotations were measured on a Perkin-Elmer 241 polarimeter.

### 5.1. General procedure for coupling of acid 5 with hydrazines and sulfonylhydrazides

A solution of acid **5** and *N*-methylmorpholine (NMM), in THF was stirred at room temperature for 30 min. Then dimethoxytriazine methylmopholinium chloride (DMTMM) was added to the reaction mixture and stirring was continued for 0.5–1.5 h. Arylhydrazine, sulfonylhydrazide as base or HCl salt were added to the reaction mixture, as a solid or THF/DMSO solution in the presence of NMM. Stirring was continued at room temperature under nitrogen until the disappearance of the starting material (TLC monitoring). After evaporation of the solvent, the crude residue was either directly purified by column chromatography, or dissolved in $CH_2Cl_2$. In the latter case, the organic layer was washed successively with 1 N aqueous HCl and 5% $NaHCO_3$ solution, dried over $Na_2SO_4$, filtered and the solvent was evaporated in vacuo. The residue was purified by column, or preparative thin-layer chromatography to afford hydrazides or sulfonylhydrazides.

#### 5.1.1. *N′*-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-4-methoxyphenylhydrazide (2a)

Prepared according to the general procedure 5.1: acid **5** (200 mg, 0.54 mmol); NMM (60 μL, 0.54 mmol); THF (5 mL); stirring at room temperature 0.5 h; DMTMM (180 mg, 0.65 mmol); time of activation 1.5 h; 4-methoxyphenylhydrazine HCl salt (283 mg, 1.62 mmol) dissolved in THF (3 mL) with NMM (180 μL, 1.62 mmol); reaction time 20 h. After extraction the crude product was purified by flash chromatography (silica gel, $CH_2Cl_2$/MeOH, 100:1 to 96:4). Yield: **2a** (124 mg, 46%). Yellowish crystals. Mp 200–201 °C; $[\alpha]_D^{21}$ −10 (*c* 0.65, DMSO); UV (MeOH, nm): 292, 282, 222, 200, 210; IR (KBr, $cm^{-1}$): 3410 (NH), 3295 (NH), 2943 (CH), 1657 (CO), 1635 (CO), 1507, 1097, 1031, 864, 825; $^1$H NMR (300 MHz, DMSO-$d_6$): 0.77–0.84 (m, 6H, CH(C$H_3$)$_2$), 1.09 (m, 1H, C$H_2$–CH(CH$_3$)$_2$), 1.41 (m, 2H, C$H_2$–C$H$(CH$_3$)$_2$), 2.17–2.27 (m, 2H, NH–CO–C$H_2$–), 2.52 (d, 3H, *J* = 3.7 Hz, CO–NH–C$H_3$), 2.74 (m, 1H, C$H$–CH$_2$–CH(CH$_3$)$_2$), 2.99–3.14 (m, 2H, CH–C$H_2$-indole), 3.62 (s, 3H, OC$H_3$), 4.47 (m, 1H, C$H$–CH$_2$-indole), 6.71 (m, 4H, H$_{aryl}$), 6.98 (m, 1H, H6$_{indole}$), 7.07 (m, 1H, H5$_{indole}$), 7.11 (s, 1H, H2$_{indole}$), 7.34 (m, 2H, N$H$NHCO, H7$_{indole}$), 7.59 (d, 1H, *J* = 7.7 Hz, H4$_{indole}$), 7.80 (q, 1H, *J* = 3.7 Hz, CO–N$H$–CH$_3$), 8.02 (d, 1H, *J* = 7.9 Hz, CON$H$), 9.63 (sl, 1H, NHN$H$–CO), 10.83 (s, 1H, NH$_{indole}$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): 22.3, 23.3, 25.4, 25.8, 27.9, 36.9, 40.6, 41.1, 53.7, 55.4, 110.5, 111.4, 113.8, 114.3, 118.3, 118.6, 120.9, 123.6, 127.6, 136.2, 143.4, 152.7, 170.8, 171.9, 174.0; MS (CI, *m/z*, %): 493.83 (M$^{+\cdot}$, 100), 463 (7), 356 (12), 242 (22); Anal. Calcd for $C_{26}H_{31}N_5O_3Cl_2{\cdot}2H_2O$: C 61.23, H 7.42, N 13.22%. Found: C 60.83, H 7.02, N 13.60%.

#### 5.1.2. *N′*-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-3,4-dichlorophenylhydrazide (2b)

Prepared according to the general procedure 5.1: acid **5** (200 mg, 0.54 mmol); NMM (60 μL, 0.54 mmol); THF (5 mL); stirring at room temperature 0.5 h; DMTMM (180 mg, 0.65 mmol); time of activation 1.5 h; 3,4-dichlorophenylhydrazine HCl salt (345 mg, 1.62 mmol) dissolved in THF (3 mL) with NMM (180 μL, 1.62 mmol); reaction time 20 h. After extraction the crude product was crystallized from ethyl acetate. Yield: **2b** (130 mg, 45%). Yellowish crystals. Mp 203–205 °C; $[\alpha]_D^{21}$ −11 (*c* 1.22, DMSO); UV (MeOH, nm): 291, 282, 274, 220, 210; IR (KBr, $cm^{-1}$): 3286 (NH), 3075 (NH), 2951 (CH), 1661 (CO), 1635 (CO), 1093, 851, 811; $^1$H NMR (300 MHz, DMSO-$d_6$): 0.77 and 0.81 (d, 2× 3H, *J* = 6.2 Hz, CH(C$H_3$)$_2$), 1.06 (m, 1H, C$H_2$–CH(CH$_3$)$_2$), 1.41 (m, 2H, C$H_2$–C$H$(CH$_3$)$_2$), 2.20 (m, 2H, NH–CO–C$H_2$–), 2.50 (d, 3H, *J* = 4.7 Hz, CO–NH–C$H_3$), 2.74 (m, 1H, C$H$–CH$_2$–CH(CH$_3$)$_2$), 3.01 (m, 2H, CH–C$H_2$-indole), 4.46 (m, 1H, C$H$–CH$_2$-indole), 6.68 (dd, 1H, *J* = 8.7, 2.5 Hz, H$_{aryl}$), 6.83 (d, 1H, *J* = 2.5 Hz, H$_{aryl}$, 6.95 (m, 1H, H6$_{indole}$), 7.02–7.09 (m, 2H, H2$_{indole}$, H5$_{indole}$), 7.32 (m, 2H, H$_{aryl}$, H7$_{indole}$), 7.55 (d, 1H, *J* = 7.7 Hz, H4$_{indole}$), 7.73 (q, 1H, *J* = 4.7 Hz, CO–N$H$–CH$_3$), 8.00 (d, 1H, *J* = 8.0 Hz, CON$H$), 8.13 (br s, 1H, N$H$NHCO), 9.73 (br s, 1H, NHN$H$CO), 10.78 (s, 1H, NH$_{indole}$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): 22.2, 23.1, 25.2, 25.6, 27.7, 36.4, 40.3, 40.9, 53.5, 110.3, 111.2, 112.5, 112.9, 118.6, 118.5, 119.1, 120.8, 123.4, 127.4, 130.3, 131.2, 136.0, 149.5, 170.8, 171.7, 173.7; MS (CI, *m/z*, %): 534.77 (M+2, 27), 533.73 (M+1, 22), 531.58 (M′+2, 100), 356 (22), 84 (46), 70 (65); Anal. Calcd for $C_{26}H_{31}N_5O_3Cl_2{\cdot}H_2O$: C 56.73, H 6.04, N 12.72%. Found: C 56.79, H 5.79, N 12.55%.

#### 5.1.3. *N′*-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-4-nitrophenylhydrazide (2c)

Prepared according to the general procedure 5.1: acid **5** (200 mg, 0.54 mmol); NMM (60 μL, 0.54 mmol); THF (5 mL); stirring at room temperature 0.5 h; DMTMM (180 mg, 0.65 mmol); time of activation 1.5 h; 4-nitrophenylhydrazine (250 mg, 1.62 mmol); reaction time 20 h. After extraction the product was crystallized from ethyl acetate and the mother-liquor was purified by preparative thin-layer chromatography (silica gel, $CH_2Cl_2$/EtOH, 98:2). Yield: **2c** (123 mg, 45%). Yellow crystals. Mp 230–232 °C; $[\alpha]_D^{21}$ +3 (*c* 0.95, DMSO); UV (MeOH, nm): 352, 291, 282, 274, 222, 210, 264; IR (KBr, $cm^{-1}$): 3286 (NH), 3084 (NH), 2952 (CH), 1665 (CO), 1635 (CO), 1516 ($NO_2$), 1331 ($NO_2$), 1111, 842; $^1$H NMR (300 MHz, DMSO-$d_6$): 0.79 and 0.83 (d, 2× 3H, *J* = 6.1 Hz, CH(C$H_3$)$_2$), 1.03–1.44 (m, 3H, C$H_2$–C$H$(CH$_3$)$_2$), 2.28 (m, 2H, NH–CO–C$H_2$–), 2.49 (d, 3H, *J* = 4.5 Hz, CO–NH–C$H_3$), 2.77 (m, 1H, C$H$–CH$_2$–CH(CH$_3$)$_2$), 3.02 (m, 2H, CH–C$H_2$-indole), 4.47 (m, 1H, C$H$–CH$_2$–indole), 6.76 (d, 2H, *J* = 9.2 Hz, H$_{aryl}$), 6.90 (m, 1H, H6$_{indole}$), 7.05 (m, 1H, H5$_{indole}$), 7.10 (s, 1H, H2$_{indole}$), 7.32 (d, 1H, *J* = 7.9 Hz, H7$_{indole}$), 7.56 (d, 1H, *J* = 7.8 Hz, H4$_{indole}$), 7.78 (q, 1H, *J* = 4.5 Hz, CO–N$H$–CH$_3$), 8.03 (d, 1H, *J* = 5.9 Hz, CON$H$), 8.05 (d, 2H, *J* = 9.2 Hz, H$_{aryl}$), 9.05 (br s, 1H, N$H$NHCO), 10.00 (br s, 1H, NHN$H$CO), 10.81 (s, 1H, NH$_{indole}$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): 22.2, 23.0, 25.2, 25.6, 27.8, 36.3, 40.4, 41.1, 53.6, 110.2, 110.5, 111.2, 118.1, 118.4, 120.8, 123.4, 125.8, 127.4, 136.0, 137.9, 154.9, 170.8, 171.7, 173.8; MS (CI, *m/z*, %): 508.88 (M$^{+\cdot}$, 100), 478 (18), 84 (50), 70 (26); Anal. Calcd for $C_{26}H_{32}N_6O_5$: C 61.40, H 6.34, N 16.52%. Found: C 61.12, H 6.16, N 16.30%.

#### 5.1.4. *N′*-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-2,4-dinitrophenylhydrazide (2d)

Prepared according to the general procedure 5.1: acid **5** (200 mg, 0.54 mmol); NMM (60 μL, 0.54 mmol); THF (5 mL); stirring at room temperature 0.5 h; DMTMM (180 mg, 0.65 mmol); time of activation 1.5 h; 2,4-dinitrophenylhydrazine (320 mg, 1.61 mmol); reaction time 20 h. After extraction the crude product was purified by flash chromatography (silica gel, $CH_2Cl_2$/MeOH, 97:3). Yield: **2d** (155 mg, 52%). Orange crystals. Mp 193–195 °C; $[\alpha]_D^{21}$ +2 (*c* 0.62, DMSO); UV (MeOH, nm): 334, 290, 282, 264, 222, 210; IR (KBr, $cm^{-1}$): 3401 (NH), 3278 (NH), 2952 (CH), 1670 (CO), 1635 (CO), 1520 ($NO_2$), 1335 ($NO_2$), 1097, 921, 829; $^1$H NMR (300 MHz, DMSO-$d_6$): 0.81 and 0.86 (d, 2× 3H, *J* = 6.0 Hz, CH(C$H_3$)$_2$), 1.16–1.49 (m, 3H, C$H_2$–C$H$(CH$_3$)$_2$), 2.35 (m, 2H, NH–CO–C$H_2$–), 2.51 (d, 3H, *J* = 4.6 Hz, CO–NH–C$H_3$), 2.82 (m, 1H, C$H$–CH$_2$–CH(CH$_3$)$_2$), 3.00 (m, 2H, CH–C$H_2$–indole), 4.48 (m, 1H, C$H$–CH$_2$–indole), 6.93 (m, 1H, H6$_{indole}$), 7.02 (m, 1H, H5$_{indole}$), 7.07 (s, 1H, H2$_{indole}$), 7.18 (d, 1H, *J* = 9.6 Hz, H$_{aryl}$), 7.26 (d, *J* = 8.0 Hz, 1H, H7$_{indole}$), 7.52 (d, *J* = 7.8 Hz, 1H, H4$_{indole}$), 7.79 (q, 1H, *J* = 4.6 Hz, CO–N$H$–CH$_3$), 8.10 (d, 1H, *J* = 8.0 Hz, CON$H$), 8.18 (dd, 1H, *J* = 9.6, 2.6 Hz, H$_{aryl}$), 8.82 (s, 1H, H$_{aryl}$), 10.05 (br s, 1H, N$H$NHCO), 10.41 (br s, 1H, NHN$H$CO), 10.72 (s, 1H, NH$_{indole}$); $^{13}$C

NMR (75 MHz, DMSO-$d_6$): 22.5, 23.1, 25.4, 25.8, 27.9, 36.5, 40.3, 41.4, 53.7, 110.4, 111.4, 115.6, 118.3, 118.5, 120.9, 123.3, 123.5, 127.5, 129.7, 129.9, 136.1, 136.7, 148.6, 170.7, 171.9, 174.0; MS (CI, *m/z*, %): 553.64 ($M^{+\cdot}$,100), 523 (17), 242 (12), 84 (64), 70 (62); Anal. Calcd for $C_{26}H_{31}N_7O_7$: C 56.41, H 5.65, N 17.71%. Found: C 56.50, H 5.65, N 17.34%.

#### 5.1.5. *N*′-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-*tert*-butoxycarbonyl-hydrazide (2e)

A solution of acid **5** (510 mg, 1.37 mmol) and *N*-methylmorpholine (164 μL, 1.50 mmol) in THF (15 mL) was stirred at room temperature for 30 min and then dimethoxytriazine methylmorpholinium chloride (DMTMM, 450 mg, 1.63 mmol) was added to the reaction mixture and stirring was continued for 1.5 h. Then Boc-hydrazine (200 mg, 1.52 mmol) was added to the reaction mixture and stirring was continued at room temperature under nitrogen overnight. After evaporation of the solvent, the crude residue was dissolved in $CH_2Cl_2$ (30 mL), the organic layer was washed at 0 °C successively with water (10 mL), 5% aq $NaHCO_3$ solution, 5% aq citric acid solution, dried over $Na_2SO_4$, filtered, and the solvent was evaporated in vacuo. The residue was purified by crystallization from ether containing 5–10% methanol. Yield: **2e** (618 mg, 92%). White crystals. Mp 162–165 °C; UV (MeOH, nm): 290, 282, 274, 229, 207; IR (KBr, $cm^{-1}$): 3387 (NH), 3279 (NH), 2946 (CH), 1712 (CO), 1632 (CO), 1542, 1246, 1161 ($SO_2$), 739; $^1$H NMR (300 MHz, acetone-$d_6$): 0.75 and 0.79 (d, 2× 3H, *J* = 6.3 Hz, CH(C$H_3$)$_2$), 1.20 (m, 1H, C$H_2$–CH(CH$_3$)$_2$), 1.42 (s, 9H, C(C$H_3$)$_3$), 1.45 (m, 2H, C$H_2$–C$H$(CH$_3$)$_2$), 2.38 (dd, 2H, *J* = 15.1, 5.6 Hz, NH–CO–C$H_2$–), 2.63 (d, 3H, *J* = 4.6 Hz, CO–NH–C$H_3$), 2.80 (m, 1H, C$H$–CH$_2$–CH(CH$_3$)$_2$), 3.24 (m, 2H, CH–C$H_2$-indole), 4.62 (m, 1H, C$H$–CH$_2$-indole), 6.99 (t, 1H, *J* = 7.4 Hz, H6$_{indole}$), 7.07 (t, 1H, *J* = 7.4 Hz, H5$_{indole}$), 7.20 (s, 1H, H2$_{indole}$), 7.35 (d, 1H, *J* = 7.4 Hz, H7$_{indole}$), 7.41–7.44 (d, 2H, *J* = 8 Hz, N$H$N$H$CO), 7.63 (d, 1H, *J* = 7.4 Hz, H4$_{indole}$), 7.97 (q, 1H, *J* = 4.6 Hz, CO–N$H$–CH$_3$), 9.14 (d, 1H, *J* = 5.6 Hz, CON$H$), 10.07 (br s, 1H, NH$_{indole}$); $^{13}$C NMR (75 MHz, acetone-$d_6$): 22.4, 23.3, 26.2, 28.3, 28.4, 37.4, 41.8, 41.9, 55.0, 80.5, 111.7, 112.0, 119.4, 119.5, 121.9, 124.3, 128.6, 137.5, 156.6, 171.9, 172.9, 175.1; Anal. Calcd for $C_{25}H_{37}N_5O_5$: C 61.58, H 7.65, N 14.37%. Found: C 61.77, H 7.88, N 14.10%.

#### 5.1.6. *N*′-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-hydrazide (2f)

A solution of Boc protected hydrazide **2e** (2.82 g, 5.79 mmol) in HCl-saturated ethanol (45 mL, C 7.7 mol/L) was stirred at room temperature for 15–30 min. After evaporation of the solvent, the crude residue was dissolved in $CH_2Cl_2$ (60 mL) and 1 M aqueous NaOH solution was added at 0 °C till pH 9. The formed solid was filtered off, washed with water (5 mL), ether (2× 5 mL), and dried. Yield: **2f** (1.46 g, 73%). White crystals. Mp 187–188 °C; IR (KBr, $cm^{-1}$): 3438 (NH), 3297 (NH), 2961 ($CH_3$), 1633 (CO), 1571 (CONH), 1026, 801; $^1$H NMR (300 MHz, DMSO-$d_6$): 0.77 (m, 6H, CH(C$H_3$)$_2$), 0.99 (m, 1H, C$H_2$–CH(CH$_3$)$_2$), 1.35 (m, 2H, C$H_2$–C$H$(CH$_3$)$_2$), 2.09 (m, 2H, NH–CO–C$H_2$–), 2.56 (d, 3H, *J* = 4.4 Hz, CO–NH–C$H_3$), 2.68 (m, 1H, C$H$–CH$_2$–CH(CH$_3$)$_2$), 3.05 (m, 2H, CH–C$H_2$-indole), 4.20 (m, 2H, N$H_2$NHCO), 4.43 (m, 1H, C$H$–CH$_2$-indole), 6.97 (m, 1H, H5$_{indole}$), 7.06 (m, 1H, H6$_{indole}$), 7.11 (s, 1H, H2$_{indole}$), 7.32 (d, 1H, *J* = 7.9 Hz, H7$_{indole}$), 7.54 (m, 1H, NH$_2$N$H$CO), 7.57 (d, 1H, *J* = 7.7 Hz, H4$_{indole}$), 7.88 (q, 1H, *J* = 4.4 Hz, CO–N$H$–CH$_3$), 7.99 (d, 1H, *J* = 7.9 Hz, CON$H$), 10.07 (br s, 1H, NH$_{indole}$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): 21.8, 22.5, 25.0, 25.2, 27.3, 36.6, 40.6, 40.8, 53.4, 110.3, 110.9, 117.8, 118.0, 120.4, 123.0, 127.5, 136.0, 169.9, 171.4, 173.7; MS (CI, *m/z*, %): 388.55 (M+1, 100), 359 (11), 357 (11), 256 (9), 219 (13); Anal. Calcd for $C_{20}H_{29}N_5O$: C 61.99, H 7.54, N 18.08%. Found: C 62.27, H 7.31, N 18.44%.

#### 5.1.7. *N*′-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-tosylhydrazide (3a)

Prepared according to the general procedure 5.1: acid **5** (150 mg, 0.40 mmol); NMM (50 μL, 0.44 mmol); THF (5 mL); stirring at room temperature 0.5 h; DMTMM (225 mg, 0.80 mmol); time of activation 1.5 h; *p*-toluenesulfonylhydrazide (375 mg, 2.01 mmol); reaction time 18 h. Purification of the crude product by column chromatography (silica gel, $CH_2Cl_2$/MeOH, 99:1). Yield: **3a** (146 mg 67%). White solid. Mp 192–193 °C; $^1$H NMR (300 MHz, DMSO-$d_6$): 0.75 (d, 6H, *J* = 6.4 Hz, CH(C$H_3$)$_2$), 0.85 (m, 1H, C$H_2$–CH(C$H_3$)$_2$), 1.25 (m, 2H, C$H_2$–C$H$(CH$_3$)$_2$), 1.99 (m, 2H, NH–CO–C$H_2$–), 2.37 (s, 3H, Ar–C$H_3$), 2.55 (d, 3H, *J* = 4.4 Hz, CO–NH–C$H_3$), 2.64 (m, 1H, C$H$–CH$_2$–CH(CH$_3$)$_2$), 3.01 (m, 2H, indole-C$H_2$–), 4.45 (m, 1H, C$H$–CH$_2$-indole), 6.98 (m, 1H, H5$_{indole}$), 7.05 (s, 1H, H2$_{indole}$), 7.07 (m, 1H, H6$_{indole}$), 7.31 (d, 1H, *J* = 7.1 Hz, H7$_{indole}$), 7.40 (d, 2H, *J* = 8.1 Hz, H$_{tosyl}$), 7.57 (d, 1H, *J* = 7.8 Hz, H4$_{indole}$), 7.75 (d, 2H, *J* = 8.1 Hz, H$_{tosyl}$), 9.80 (br s, 1H, NH), 10.05 (br s, 1H, NH), 10.80 (br s, 1H, NH$_{indole}$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): 21.3, 22.0, 25.3, 25.8, 27.9, 36.5, 39.9, 43.1, 53.5, 110.5, 111.4, 118.3, 118.6, 121.0, 123.5, 127.5, 127.9, 129.5, 136.2, 143.3, 150.1, 169.6, 171.9, 173.7.

#### 5.1.8. *N*′-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-4-chlorobenzene-sulfonylhydrazide (3b)

Prepared according to the general procedure 5.1: acid **5** (500 mg, 1.34 mmol); NMM (162 μL, 1.47 mmol); THF (30 mL); stirring at room temperature 0.5 h; DMTMM (484 mg, 1.61 mmol); time of activation 0.5 h; *p*-chlorophenylsulfonylhydrazide HCl salt (980 mg, 4.02 mmol) dissolved in THF (15 mL) with NMM (442 μL, 4.02 mmol); reaction time 20 h. After extraction the crude product was purified by column chromatography (silica gel, heptane/AcOEt, 70:30). Yield: **3b** (82 mg, 11%) after crystallization from $CH_2Cl_2$. White-yellowish solid. Mp 217–219 °C; $[\alpha]_D^{21}$ −5 (*c* 2.54, DMSO); UV (MeOH, nm): 291, 282, 275, 227, 210; IR (KBr, $cm^{-1}$): 3396 (NH), 3281 (NH), 2947 (CH), 1633 (CO), 1595 (CO), 1538, 1338 ($SO_2$), 1162 ($SO_2$), 1010, 886, 743; $^1$H NMR (300 MHz, $CD_3OD$): 0.73 and 0.77 (d, 2× 3H, *J* = 6.4 Hz, CH(C$H_3$)$_2$), 0.87 (m, 1H, C$H_2$–CH(CH$_3$)$_2$), 1.31 (m, 2H, C$H_2$–C$H$(CH$_3$)$_2$), 2.11 (m, 2H, NH–CO–C$H_2$–), 2.56 (m, 3H, CO–NH–C$H_3$), 2.57 (m, 1H, C$H$–CH$_2$–CH(CH$_3$)$_2$), 3.18 (m, 2H, CH–C$H_2$-indole), 4.53 (m, 1H, C$H$–CH$_2$-indole), 6.98 (m, 1H, H6$_{indole}$), 7.05 (s, 1H, H2$_{indole}$), 7.07 (m, 1H, H5$_{indole}$), 7.31 (d, 1H, *J* = 8.0 Hz, H7$_{indole}$), 7.52 (d, 2H, *J* = 8.6 Hz, H$_{aryl}$), 7.57 (d, 1H, *J* = 7.8 Hz, H4$_{indole}$), 7.85 (d, 2H, *J* = 8.6 Hz, H$_{aryl}$); $^{13}$C NMR (75 MHz, $CD_3OD$): 22.3, 23.4, 26.3, 26.7, 28.7, 37.3, 42.2, 42.3, 55.7, 111.2, 112.3, 119.4, 119.7, 122.4, 124.5, 128.7, 130.3, 131.0, 138.0, 138.8, 140.6, 172.1, 174.4, 176.8; MS (CI, *m/z*, %): 562.53 (M+1, 100), 531 (13), 375 (24), 84 (37); Anal. Calcd for $C_{26}H_{32}ClN_5O_5S{\cdot}0.5H_2O$: C 54.68, H 5.82, N 12.26%. Found: C 54.31, H 5.93, N 11.91%.

#### 5.1.9. *N*′-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-2,4,6-*tri*-*iso*propyl-phenylsulfonylhydrazide (3c)

Prepared according to the general procedure 5.1: acid **5** (147 mg, 0.39 mmol); NMM (40 μL, 0.36 mmol); THF (5 mL); stirring at room temperature 0.5 h; DMTMM (135 mg, 0.49 mmol); time of activation 1.0 h; 2,4,6-tri-*i*-propylphenylsulfonylhydrazide (110 mg, 0.37 mmol); reaction time 1.5 h. After extraction the crude product was purified by preparative thin-layer chromatography (silica gel, $CH_2Cl_2$/EtOH, 98:2). Yield: **3c** (65 mg, 27%). White solid. Mp 98–100 °C; $[\alpha]_D^{21}$ −16 (*c* 0.44, DMSO); UV (MeOH, nm): 291, 281, 272, 220, 210; IR (KBr, $cm^{-1}$): 3330 (NH), 3260 (NH), 2952 (CH), 1683 (CO), 1639 (CO), 1529, 1459, 1326 ($SO_2$), 1155 ($SO_2$), 1098, 882; $^1$H NMR (300 MHz, DMSO-$d_6$): 0.70 and 0.74 (d, 2× 3H, *J* = 5.8 Hz, CH(C$H_3$)$_2$), 0.87 (m, 1H, C$H_2$–CH(CH$_3$)$_2$),

1.21 (m, 20H, $CH_2$–$CH(CH_3)_2$), 3× ($CH(CH_3)_{2aryl}$), 2.00–2.18 (m, 2H, NH–CO–$CH_2$–), 2.52 (d, 3H, $J$ = 5.2 Hz, CO–NH–$CH_3$), 2.62 (m, 1H, $CH$–$CH_2$–$CH(CH_3)_2$), 2.89–3.20 (m, 3H, CH–$CH_2$-indole, $CH(CH_3)_{2aryl}$), 4.07 (m, 2H, $CH(CH_3)_{2aryl}$), 4.38 (m, 1H, $CH$–$CH_2$-indole), 6.93–7.08 (m, 3H, $H6_{indole}$, $H5_{indole}$, $H2_{indole}$), 7.21 (s, 2H, $H_{aryl}$), 7.32 (d, 1H, $J$ = 8.0 Hz, $H7_{indole}$), 7.54 (d, 1H, $J$ = 7.6 Hz, $H4_{indole}$), 7.71 (q, 1H, $J$ = 5.2 Hz, CO–N$H$–$CH_3$), 7.97 (d, 1H, $J$ = 8.0 Hz, CON$H$), 9.47 (br s, 1H, NH), 9.99 (br s, 1H, NH), 10.76 (br s, 1H, $NH_{indole}$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): 22.2, 23.3, 23.6, 25.0, 25.4, 25.8, 27.7, 29.7, 33.5, 36.3, 39.2, 42.6, 53.6, 110.6, 111.4, 118.3, 118.6, 121.0, 120.9, 121.0, 127.4, 133.0, 136.2, 150.9, 152.4, 170.2, 171.9, 173.9; MS (CI, *m/z*, %): 675.97 (M+Na, 100), 653.95 (M, 50), 623 (12), 356 (9), 55 (34), 43 (39); Anal. Calcd for $C_{35}H_{51}N_5O_5S{\cdot}0.5H_2O$: C 63.42, H 7.91, N 10.56%. Found: C 63.28, H 7.64, N 10.51%.

#### 5.1.10. *N*′-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-methanesulfonyl-hydrazide (3d)

Prepared according to the general procedure 5.1: acid **5** (600 mg, 1.61 mmol); NMM (200 μL, 1.77 mmol); THF (35 mL); stirring at room temperature 1.5 h; DMTMM (534 mg, 1.93 mmol); time of activation 0.5 h; methanesulfonylhydrazide HCl salt (1.18 g, 8.05 mmol) dissolved in DMSO (20 mL) with NMM (890 μL, 8.09 mmol); reaction time 20 h. Purification of the crude product by column chromatography (silica gel, $CH_2Cl_2$/MeOH, 99.5:0.5). Yield: **3d** (231 mg, 31%) as a yellowish oil which was crystallized from $CH_2Cl_2$. White-yellowish solid. Mp 179–181 °C; $[\alpha]_D^{21}$ −6 (*c* 1.21, MeOH); UV (MeOH, nm): 291, 282, 218, 205; IR (KBr, $cm^{-1}$): 3836 (NH), 3281 (NH), 2937 ($CH_3$), 1633 (CO), 1538, 1329 ($SO_2$), 1157 ($SO_2$), 967, 867; $^1$H NMR (300 MHz, acetone-$d_6$): 0.77 and 0.82 (d, 2× 3H, $J$ = 6.1 Hz, $CH(CH_3)_2$), 1.06 (m, 1H, $CH_2$–$CH(CH_3)_2$), 1.39 (m, 2H, $CH_2$–$CH(CH_3)_2$), 2.19 (m, 2H, NH–CO–$CH_2$–), 2.55 (d, 3H, $J$ = 4.6 Hz, $CH_3$NHNH–), 2.73 (m, 1H, $CH$–$CH_2$–$CH(CH_3)_2$), 2.86 (s, 3H, $CH_3SO_2NH$), 3.02 (m, 2H, CH–$CH_2$-indole), 4.45 (m, 1H, $CH$–$CH_2$-indole), 6.98 (m, 1H, $H6_{indole}$), 7.06 (m, 1H, $H5_{indole}$), 7.12 (d, 1H, $J$ = 1.7 Hz, $H2_{indole}$), 7.33 (d, 1H, $J$ = 7.9 Hz, $H7_{indole}$), 7.57 (d, 1H, $J$ = 7.7 Hz, $H4_{indole}$), 7.81 (d, 1H, $J$ = 4.7 Hz, NH), 8.04 (d, 1H, $J$ = 8.1 Hz, CONH), 9.40 (s, 1H, NH), 10.13 (s, 1H, $NHSO_2$), 10.80 (s, 1H, $NH_{indole}$); $^{13}$C NMR (75 MHz, acetone-$d_6$): 22.4, 23.1, 25.3, 25.8, 27.9, 36.2, 40.0, 40.3, 41.1, 53.6, 110.5, 111.4, 118.3, 118.6, 121.0, 123.6, 127.6, 136.2, 170.8, 171.9, 173.9; MS (CI, *m/z*, %): 466.57 (M+1, 100), 436 (18), 375 (16), 357 (8), 84 (27), 80 (17); Anal. Calcd for $C_{21}H_{31}N_5O_5S$: C 54.18, H 6.71, N 15.04%. Found: C 54.17, H 6.60, N 14.60%.

### 5.2. General procedure for the sulfonylation of hydrazide 2f

Sulfonylchloride (pure or in THF solution) was added at 0 °C to a solution of hydrazide **2f** in THF containing co-solvents (EtOH or DMF or DMSO) and a base ($NaHCO_3$ or pyridine or $Et_3N$). Stirring was continued at room temperature until the disappearance of the starting material (TLC monitoring). After evaporation of the solvent, the residue was dissolved in $CH_2Cl_2$ and the organic layer was washed with acid (5% aq HCl or 5% aq citric acid) solution, then with 5% aq $NaHCO_3$, dried over $Na_2SO_4$, filtered, and evaporated to dryness. In some cases final product precipitated during extraction and was filtered off. Purification of the products was achieved by crystallization or column chromatography.

#### 5.2.1. *N*′-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-4-nitrobenzene-sulfonylhydrazide (3e)

Prepared according to the general procedure 5.2: hydrazide **2f** (300 mg, 0.78 mmol); solvents: THF (7 mL) and EtOH (4 mL); base: $NaHCO_3$ (655 mg, 7.8 mmol); 4-nitrobenzenesulfonylchloride (172 mg, 0.78 mmol); reaction time 18 h. After extraction, the crude product was purified by column chromatography (silica gel, $CH_2Cl_2$/acetone, 50:50). Yield: **3e** (45 mg, 11%). Yellow crystals. Mp 117–119 °C; $[\alpha]_D^{21}$ −11 (*c* 0.092, DMSO); UV (MeOH, nm): 291, 281, 272, 220, 210; IR (KBr, $cm^{-1}$): 3401 (NH), 3330 (NH), 2934 (CH), 1674 (CO), 1652 (CO), 1533, 1344 ($SO_2$), 1164 ($SO_2$), 1089, 851; $^1$H NMR (300 MHz, DMSO-$d_6$): 0.69–0.81 (m, 7H, $CH(CH_3)_2$, $CH_2$–$CH(CH_3)_2$), 1.20–1.33 (m, 2H, $CH_2$–$CH(CH_3)_2$, $CH_2$–$CH(CH_3)_2$), 2.02 (m, 2H, NH–CO–$CH_2$–), 2.50–2.58 (m, 4H, CO–NH–$CH_3$, $CH$–$CH_2$–$CH(CH_3)_2$), 2.87–3.10 (m, 2H, CH–$CH_2$–indole), 4.42 (m, 1H, $CH$–$CH_2$-indole), 6.93–7.14 (m, 3H, $H6_{indole}$, $H5_{indole}$, $H2_{indole}$), 7.33 (d, 1H, $J$ = 8.1 Hz, $H7_{indole}$), 7.55 (d, 1H, $J$ = 7.8 Hz, $H4_{indole}$), 7.73 (q, 1H, $J$ = 4.6 Hz, CO–N$H$–$CH_3$), 7.96 (d, 1H, $J$ = 8.1 Hz, CONH), 8.08 (d, 2H, $J$ = 8.7 Hz, $H_{aryl}$), 8.39 (d, 2H, $J$ = 8.7 Hz, $H_{aryl}$), 10.17 (br s, 2H, 2NH), 10.80 (br s, 1H, $NH_{indole}$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): 22.0, 23.3, 25.3, 25.8, 27.9, 36.3, 38.8, 40.6, 53.6, 110.5, 111.4, 118.3, 118.6, 121.0, 123.6, 124.3, 127.5, 129.6, 136.2, 145.3, 150.0, 170.0, 171.9, 173.6; MS (CI, *m/z*, %): 573.64 (M+1, 100), 543 (12), 160 (3).

#### 5.2.2. *N*′-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-2-nitrobenzene-sulfonylhydrazide (3f)

Prepared according to the general procedure 5.2: hydrazide **2f** (600 mg, 1.55 mmol); solvents: THF (10 mL) and EtOH (10 mL); base: pyridine (140 μL, 1.71 mmol); 2-nitrobenzenesulfonylchloride (344 mg, 1.55 mmol); reaction time 18 h. Purified by column chromatography (silica gel, $CH_2Cl_2$/MeOH, 95:5). Yield: **3f** (525 mg, 59%). Yellow crystals. Mp 94–95 °C; $[\alpha]_D^{21}$ −0.3 (*c* 1.82, DMSO); UV (MeOH, nm): 291, 281, 272, 220, 210; IR (KBr, $cm^{-1}$): 3286 (NH), 2952 (CH), 1696 (CO), 1639 (CO), 1542, 1353 ($SO_2$), 1168 ($SO_2$), 851, 781; $^1$H NMR (300 MHz, DMSO-$d_6$): 0.67–0.71 (m, 6H, $CH(CH_3)_2$), 0.79 (m, 1H, $CH_2$–$CH(CH_3)_2$), 1.22 (m, 2H, $CH_2$–$CH(CH_3)_2$, $CH_2$–$CH(CH_3)_2$), 2.05 (m, 2H, NH–CO–$CH_2$–), 2.48 (m, 4H, CO–NH–$CH_3$, $CH$–$CH_2$–$CH(CH_3)_2$), 2.87–3.15 (m, 2H, CH–$CH_2$-indole), 4.40 (m, 1H, $CH$–$CH_2$-indole), 6.89–7.09 (m, 3H, $H6_{indole}$, $H5_{indole}$, $H2_{indole}$), 7.28 (d, 1H, $J$ = 7.8 Hz, $H7_{indole}$), 7.51 (d, 1H, $J$ = 7.5 Hz, $H4_{indole}$), 7.70–8.05 (m, 6H, $H_{aryl}$, CO–N$H$–$CH_3$, CON$H$), 10.17 (br s, 2H, N$H$N$H$), 10.76 (br s, 1H, $NH_{indole}$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): 22.1, 23.3, 25.3, 25.8, 27.8, 36.3, 38.9, 40.5, 53.6, 110.5, 111.4, 118.3, 118.6, 121.0, 123.6, 124.5, 127.5, 131.0, 132.1, 132.6, 134.7, 136.2, 147.9, 170.2, 171.9, 173.7; Anal. Calcd for $C_{26}H_{32}N_6O_7S{\cdot}H_2O$: C 52.87, H 5.80, N 14.23%. Found: C 52.86, H 5.37, N 14.12%.

#### 5.2.3. *N*′-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-dodecansulfonyl-hydrazide (3g)

Prepared according to the general procedure 5.2: Hydrazide **2f** (100 mg, 0.258 mmol); solvents: THF (3 mL) and DMSO (0.4 mL); base: pyridine (23 μL, 0.284 mmol); dodecansulfonylchloride (69 mg, 0.258 mmol) dissolved in THF (2 mL); reaction time 24 h. After extraction, the crude product was purified by column chromatography (silica gel, $CH_2Cl_2$/methanol, 95:5). Yield: **3g** (55 mg, 34%). White crystals. Mp 171–173 °C; $[\alpha]_D^{21}$ −8 (*c* 0.50, DMSO); UV (MeOH, nm): 290, 283, 274, 222, 206; IR (KBr, $cm^{-1}$): 3401 (NH), 3339 (NH), 2916 (CH), 1661 (CO), 1635 (CO), 1538, 1454, 1335 ($SO_2$), 1142 ($SO_2$), 741; $^1$H NMR (300 MHz, acetone-$d_6$, DMSO-$d_6$): 0.76–0.89 (m, 9H, $CH(CH_3)_2$, $CH_2$–$CH_3$), 1.09 (m, 1H, $CH_2$–$CH(CH_3)_2$), 1.24 (m, 22H, –$CH_2$–), 1.78 (m, 2H, $CH_2$–$CH(CH_3)_2$), 2.25 (m, 2H, NH–CO–$CH_2$–), 2.58 (d, 3H, $J$ = 4.6 Hz, CO–NH–$CH_3$), 2.75 (m, 1H, $CH$–$CH_2$–$CH(CH_3)_2$), 2.98–3.18 (m, 2H, CH–$CH_2$-indole), 4.52 (m, 1H, $CH$–$CH_2$-indole), 6.98 (m, 1H, $H5_{indole}$), 7.06 (m, 1H, $H6_{indole}$), 7.14 (s, 1H, $H2_{indole}$), 7.34 (d, 1H, $J$ = 7.9 Hz, $H7_{indole}$), 7.60 (d, 1H, $J$ = 7.8 Hz, $H4_{indole}$), 7.75 (q, 1H, $J$ = 4.6 Hz, CO–N$H$–$CH_3$), 7.97 (d, 1H, $J$ = 8.2 Hz, CONH), 9.33 (br s, 1H, $SO_2$N$H$–NHCO), 10.11 (br s, 1H, $SO_2$NH–N$H$CO), 10.78 (br s, 1H,

$NH_{indole}$); $^{13}C$ NMR (75 MHz, acetone-$d_6$, DMSO-$d_6$): 14.0, 22.3, 22.4, 23.2, 23.3, 24.8, 25.0, 28.1, 28.2, 29.3, 29.5, 30.0, 30.3, 31.6, 36.3, 40.5, 40.7, 51.6, 53.7, 110.6, 111.4, 118.3, 118.6, 120.9, 123.6, 127.7, 136.4, 170.8, 172.0, 173.8; MS (CI, *m/z*, %): 620.40 (M+1, 48), 130 (10), 83 (100); Anal. Calcd for $C_{32}H_{53}N_5O_5S$: C 62.01, H 8.62, N 11.30%. Found: C 61.65, H 8.57, N 11.06%.

#### 5.2.4. *N*′-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-hexadecansulfonylhydrazide (3h)

Prepared according to the general procedure 5.2: hydrazide **2f** (150 mg, 0.388 mmol); solvents: THF (3 mL) and DMSO (0.2 mL); base: $NaHCO_3$ (65 mg, 0.776 mmol); hexadecansulfonylchloride (126 mg, 0.388 mmol) dissolved in THF (2 mL); reaction time 6 h. After extraction, the crude product was purified by column chromatography (silica gel, $CH_2Cl_2$/methanol, 97:3) and then crystallized from ether. Yield: **3h** (65 mg, 25%). White crystals. Mp 149–152 °C; $[\alpha]_D^{21}$ −5 (*c* 0.90, DMSO); UV (MeOH, nm): 291, 282, 273, 221, 206; IR (KBr, $cm^{-1}$): 3392 (NH), 3277 (NH), 2916 (CH), 2846, 1657 (CO), 1635 (CO), 1542, 1454, 1335 ($SO_2$), 1146 ($SO_2$), 1098, 877, 741; $^1H$ NMR (300 MHz, DMSO-$d_6$): 0.75–0.89 (m, 9H, CH($CH_3$)$_2$, $CH_2$–$CH_3$), 1.01 (m, 1H, $CH_2$–$CH$($CH_3$)$_2$), 1.25 (m, 30H, –$CH_2$–), 1.73 (m, 2H, $CH_2$–CH($CH_3$)$_2$), 2.10–2.24 (m, 2H, NH–CO–$CH_2$–), 2.55 (d, 3H, *J* = 2.5 Hz, CO–NH–$CH_3$), 2.71 (m, 1H, $CH$–$CH_2$–CH($CH_3$)$_2$), 2.92–3.14 (m, 2H, CH–$CH_2$-indole), 4.46 (m, 1H, $CH$–$CH_2$-indole), 6.97 (m, 1H, $H5_{indole}$), 7.05 (m, 1H, $H6_{indole}$), 7.11 (s, 1H, $H2_{indole}$), 7.32 (d, 1H, *J* = 8.0 Hz, $H7_{indole}$), 7.57 (d, 1H, *J* = 7.8 Hz, $H4_{indole}$), 7.81 (q, 1H, *J* = 2.5 Hz, CO–N$H$–$CH_3$), 8.06 (d, 1H, *J* = 8.3 Hz CONH), 9.40 (br s, 1H, $SO_2N$*H*–NHCO), 10.09 (br s, 1H, $SO_2$NH–N*H*CO), 10.81 (br s, 1H, $NH_{indole}$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$): 14.2, 22.3, 22.4, 23.2, 23.3, 25.4, 25.8, 28.0, 28.9, 29.0, 29.1, 29.3, 31.5, 36.4, 40.5, 41.0, 51.5, 53.7, 110.5, 111.4, 118.3, 118.7, 121.0, 123.6, 127.6, 136.2, 170.7, 171.9, 173.8; MS (CI, *m/z*, %): 676.50 (M+1, 100), 645 (9), 120 (10); Anal. Calcd for $C_{36}H_{53}N_5O_5S{\cdot}4H_2O$: C 57.80, H 9.31, N 9.36%. Found: C 57.77, H 8.96, N 9.56%.

#### 5.2.5. *N*′-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-4-phenoxybenzenesulfonylhydrazide (3i)

Prepared according to the general procedure 5.2: hydrazide **2f** (203 mg, 0.525 mmol); solvents: THF (3 mL) and DMF (3 mL); base: triethylamine (90 μL, 0.646 mmol); 4-phenoxybenzenesulfonylchloride (163 mg, 0.607 mmol); reaction time 5 h. After extraction, the crude product was purified by column chromatography (silica gel, $CH_2Cl_2$/MeOH, 99:1 to 93:7). Yield: **3i** (70 mg, 18%). Yellowish crystals. Mp 107–109 °C; $[\alpha]_D^{21}$ −9 (*c* 0.24, DMSO); UV (MeOH, nm): 291, 281, 271, 220; IR (KBr, $cm^{-1}$): 3401 (NH), 3286 (NH), 2943 (CH), 1670 (CO), 1635 (CO), 1582, 1335 ($SO_2$), 1155 ($SO_2$), 1089, 864, 833, 798, 741; $^1H$ NMR (300 MHz, $CD_3OD$): 0.73 and 0.77 (d, 2× 3H, *J* = 6.3 Hz, CH($CH_3$)$_2$), 0.97 (m, 1H, $CH_2$–CH($CH_3$)$_2$), 1.30 (m, 2H, $CH_2$–CH($CH_3$)$_2$, $CH_2$–$CH$($CH_3$)$_2$), 2.13 (m, 2H, NH–CO–$CH_2$–), 2.59 (m, 4H, CO–NH–$CH_3$, $CH$–$CH_2$–CH($CH_3$)$_2$), 3.10–3.30 (m, 2H, CH–$CH_2$-indole), 4.54 (m, 1H, $CH$–$CH_2$-indole), 6.90–7.90 (m, 14H, $H_{aryl}$, $H_{indole}$); $^{13}C$ NMR (75 MHz, $CD_3OD$): 22.4, 23.4, 26.4, 26.7, 28.7, 37.3, 42.0, 42.1, 55.7, 111.2, 112.3, 118.4, 119.4, 119.7, 121.4, 122.4, 124.5, 126.1, 128.8, 131.3, 131.7, 133.3, 138.0, 156.6, 163.5, 172.1, 174.4, 176.8; MS (CI, *m/z*, %): 620.63 (M+1, 100), 590 (9), 379 (6); Anal. Calcd for $C_{31}H_{37}N_5O_6S{\cdot}2H_2O$: C 58.61, H 6.30, N 10.68%. Found: C 58.55, H 5.85, N 10.59%.

#### 5.2.6. *N*′-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-2-naphthalenesulfonylhydrazide (3j)

Prepared according to the general procedure 5.2: hydrazide **2f** (200 mg, 0.517 mmol); solvents: THF (5 mL) and DMF (5 mL); base: triethylamine (87 μL, 0.62 mmol); 2-naphthalenesulfonylchloride (130 mg, 0.573 mmol); reaction time 3 h. During extraction the product precipitated and the mother-liquor was also purified by column chromatography (silica gel, $CH_2Cl_2$/MeOH, 98:2 to 90:10). Yield: **3j** (70 mg, 23%). Yellowish crystals. Mp 87–89 °C; $[\alpha]_D^{21}$ −12 (*c* 1.38, DMSO); UV (MeOH, nm): 291, 281, 272, 220, 210; IR (KBr, $cm^{-1}$): 3401 (NH), 3357 (NH), 2952 (CH), 1683 (CO), 1635 (CO), 1542, 1335 ($SO_2$), 1164 ($SO_2$), 1128, 860, 816; $^1H$ NMR (300 MHz, $CD_3OD$): 0.63 (m, 6H, CH($CH_3$)$_2$), 0.81 (m, 1H, $CH_2$–CH($CH_3$)$_2$), 1.20 (m, 2H, $CH_2$–CH($CH_3$)$_2$, $CH_2$–$CH$($CH_3$)$_2$), 2.07 (m, 2H, NH–CO–$CH_2$–), 2.45 (m, 1H, $CH$–$CH_2$–CH($CH_3$)$_2$), 2.50 (d, 3H, *J* = 4.4 Hz, CO–NH–$CH_3$), 3.00–3.20 (m, 2H, CH–$CH_2$-indole), 4.45 (m, 1H, $CH$–$CH_2$-indole), 6.96 (m, 1H, $H6_{indole}$), 7.00 (s, 1H, $H2_{indole}$), 7.06 (m, 1H, $H5_{indole}$), 7.30 (d, 1H, *J* = 8.1 Hz, $H7_{indole}$), 7.53 (d, 1H, *J* = 7.8 Hz, $H4_{indole}$), 7.57–8.06 (m, 11H, $H_{aryl}$, NH), 8.46 (br s, 1H, $NH_{indole}$); $^{13}C$ NMR (75 MHz, $CD_3OD$): 22.2, 23.3, 26.2, 26.6, 28.6, 37.3, 42.1, 42.2, 55.7, 111.2, 112.2, 119.4, 119.7, 122.4, 124.5, 128.6, 128.7, 129.0, 130.1, 130.2, 130.4, 130.8, 136.6, 136.9, 138.0, 172.1, 174.3, 176.8; MS (CI, *m/z*, %): 578.62 (M+1, 100), 548 (14), 350 (5); Anal. Calcd for $C_{30}H_{35}N_5O_5S{\cdot}0.5H_2O$: C 61.42, H 6.18, N 11.94%. Found: C 61.55, H 5.87, N 11.89%.

#### 5.2.7. *N*′-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-(4-succinimidyl)-benzenesulfonylhydrazide (3l)

Prepared according to the general procedure 5.2: hydrazide **2f** (40 mg, 0.10 mmol); solvents: THF (4 mL) and DMSO (0.4 mL); base: pyridine (10 μL, 0.11 mmol); 4-succinimidylbenzenesulfonylchloride (28 mg, 0.10 mmol) dissolved in THF (3 mL); reaction time 2 h. After extraction, the crude product was crystallized in ether containing 10% methanol. Yield: **3l** (15 mg, 63%). Pale yellowish crystals. Mp 210–212 °C; $[\alpha]_D^{21}$ −5.5 (*c* 1.54, DMSO); UV (MeOH, nm): 290, 282, 276, 222, 210; IR (KBr, $cm^{-1}$): 3721 (NH), 3291 (NH), 2947 (CH), 1710 (CO), 1638 (CO), 1533, 1338 ($SO_2$), 1162 ($SO_2$), 1081, 1005, 757; $^1H$ NMR (300 MHz, acetone-$d_6$): 0.75 and 0.80 (d, 2× 3H, *J* = 6.1 Hz, CH($CH_3$)$_2$), 1.06 (m, 1H, $CH_2$–CH($CH_3$)$_2$), 1.49 (m, 2H, $CH_2$–CH($CH_3$)$_2$, $CH_2$–$CH$($CH_3$)$_2$), 2.19–2.53 (m, 2H, NH–CO–$CH_2$–), 2.60 (d, 3H, *J* = 4.6 Hz, CO–NH–$CH_3$), 2.87 (m, 4H, CO$CH_2$–$CH_2$CO), 2.90 (m, 1H, $CH$–$CH_2$–CH($CH_3$)$_2$), 3.15 (m, 2H, CH–$CH_2$-indole), 4.52 (m, 1H, $CH$–$CH_2$-indole), 6.99–7.19 (m, 4H, $H2_{indole}$, $H5_{indole}$, $H6_{indole}$, CONH), 7.37 (d, 1H, *J* = 7.9 Hz, $H7_{indole}$), 7.51 (d, 2H, *J* = 8.5 Hz, $H_{aryl}$), 7.61 (d, 1H, *J* = 7.7 Hz, H4), 7.99 (d, 2H, *J* = 8.5 Hz, $H_{aryl}$), 8.67 (m, 1H, CO–N$H$–$CH_3$), 9.58 (br s, 1H, NH), 10.00 (br s, 2H, NH, $NH_{indole}$); $^{13}C$ NMR (75 MHz, acetone-$d_6$): 22.6, 23.2, 26.2, 26.3, 28.3, 29.3, 36.8, 41.6, 42.1, 54.8, 111.8, 112.0, 119.4, 119.5, 122.0, 124.5, 127.7, 128.7, 129.7, 137.5, 138.0, 138.5, 170.9, 172.6, 174.8, 177.2; MS (CI, *m/z*, %): 625.53 (M+1, 100), 595 (8), 228 (8); Anal. Calcd for $C_{30}H_{36}N_6O_7S$: C 57.68, H 5.81, N 13.45%. Found: C 57.02, H 5.75, N 12.87%.

#### 5.2.8. *N*′-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-(5-methylhexanoyl)}-*N*-4-(4-bromophenyl)-benzenesulfonylhydrazide (3m)

Prepared according to the general procedure 5.2: hydrazide **2f** (510 mg, 1.32 mmol); solvents: THF (8 mL) and EtOH (8 mL); base: pyridine (117 μL, 1.45 mmol); 4-(4-bromophenyl)benzenesulfonylchloride (437 mg, 1.32 mmol); reaction time 18 h. During extraction the product precipitated. Yield: **3m** (630 mg, 70%). Pale yellowish crystals. Mp 126–127 °C; $[\alpha]_D^{21}$ −1.5 (*c* 0.73, DMSO); UV (MeOH, nm): 291, 281, 272, 220, 210; IR (KBr, $cm^{-1}$): 3436 (NH), 3269 (NH), 2908 (CH), 1657 (CO), 1635 (CO), 1542, 1370 ($SO_2$), 1168 ($SO_2$), 1080, 811, 776; $^1H$ NMR (300 MHz, DMSO-$d_6$): 0.68 (m, 6H, CH($CH_3$)$_2$), 0.79 (m, 1H, $CH_2$–CH($CH_3$)$_2$), 1.24 (m, 2H, $CH_2$–CH($CH_3$)$_2$, $CH_2$–$CH$($CH_3$)$_2$), 1.80–2.01 (m, 2H, NH–CO–$CH_2$–), 2.50 (m, 4H, CO–NH–$CH_3$, $CH$–$CH_2$–CH($CH_3$)$_2$), 2.90–3.18 (m, 2H, CH–$CH_2$-indole), 4.40 (m, 1H, $CH$–$CH_2$-indole), 6.93–7.94 (m, 15H,

$H_{aryl}$, $H_{indole}$, CONH, (CONHCH$_3$), 9.91 (br s, 1H, $SO_2NHNHCO$), 10.03 (s, 1H, $SO_2NHNH$), 10.77 (s, 1H, $NH_{indole}$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): 22.0, 23.4, 25.3, 25.8, 27.9, 36.5, 39.7, 40.5, 53.5, 110.5, 111.4, 118.3, 118.6, 121.0, 122.4, 123.5, 127.5, 126.2, 126.5, 127.0, 126.6, 129.0, 129.3, 132.0, 132.2, 136.2, 137.8, 143.1, 148.0, 170.0, 171.9, 173.7; MS (CI, *m/z*, %): 682.54 ($M^{+\cdot}$, 100), 389 (12), 289 (9), 248 (9); Anal. Calcd for $C_{32}H_{36}BrN_5O_5S\cdot H_2O$: C 54.86, H 5.47, N 9.99%. Found: C 54.96, H 5.71, N 9.59%.

### 5.3. *N*′-{3-(*R*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-2-aminobenzenesulfonyl-hydrazide (3k)

A solution of **3f** (470 mg, 0.82 mmol) in ethanol (10 mL) was hydrogenated at atmospheric pressure in the presence of 10% palladium on charcoal (45 mg) for 2–3 h. After filtration of the catalyst, the solvent was evaporated and the residue was crystallized from methanol giving **3k** (252 mg, 57%), as white crystals. Mp 217–220 °C; $[\alpha]_D^{21}$ −6 (*c* 0.68, DMSO); IR (KBr, cm$^{-1}$): 3358 (NH), 2933 (CH), 1689 (CO), 1637 (CO), 1522, 1323 ($SO_2$), 1178 ($SO_2$); $^1$H NMR (300 MHz, DMSO-$d_6$): 0.73–0.77 (m, 6H, CH(C$H_3$)$_2$), 0.81 (m, 1H, C$H_2$–CH(CH$_3$)$_2$), 1.27 (m, 2H, C$H_2$–CH(CH$_3$)$_2$, CH$_2$–C$H$(CH$_3$)$_2$), 2.03 (m, 2H, NH–CO–C$H_2$–), 2.52 (m, 4H, CO–NH–C$H_3$, C$H$–CH$_2$–CH(CH$_3$)$_2$), 2.93 (m, 2H, CH–C$H_2$-indole), 4.42 (m, 1H, C$H$–CH$_2$-indole), 6.06 (br s, 2H, Ph–N$H_2$), 6.55 (m, 1H, $H_{aryl}$), 6.96 (m, 1H, H6$_{indole}$), 7.06 (m, 2H, H5$_{indole}$, H2$_{indole}$), 7.25 (m, 1H, $H_{aryl}$), 7.33 (d, 1H, *J* = 7.8 Hz, H7$_{indole}$), 7.46 (d, 1H, *J* = 7.8 Hz, $H_{aryl}$), 7.56 (d, 1H, *J* = 7.7 Hz, H4$_{indole}$), 7.75 (d, 1H, *J* = 4.0 Hz, CO–N$H$–CH$_3$), 7.93 (d, 1H, *J* = 8.0 Hz, CON$H$), 9.51 (br s, 1H, NH), 9.94 (br s, 1H, NH), 10.78 (br s, 1H, $NH_{indole}$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): 22.1, 23.4, 25.4, 25.8, 27.9, 36.5, 40.3, 40.5, 53.6, 110.5, 111.4, 114.8, 117.1, 117.8, 118.4, 118.6, 121.0, 123.5, 127.5, 130.2, 134.3, 136.2, 147.7, 169.9, 172.0, 173.8; MS (CI, *m/z*, %): 542.6 ($M^{+\cdot}$, 100), 512 (50), 356 (18), 242 (12), 218 (7); Anal. Calcd for $C_{26}H_{34}N_6O_5S\cdot H_2O$: C 55.70, H 6.47, N 14.99%. Found: C 55.65, H 6.14, N 14.89%.

### 5.4. General procedure for the condensation of carboxylic acids 7, 9v,w with (*S*)-tryptophan-*N*-methylamide (6) or (*S*)-2-phenyltryptophan-*N*-methylamide (8)

A mixture of acid **7** or **9v** or **9w**, *N*-methylmorpholine (NMM), and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride) (DMTMM) in dry THF was stirred at room temperature for 30 min and then (*S*)-tryptophan-*N*-methylamide (**6**) or (*S*)-2-phenyltryptophan-*N*-methylamide (**8**) was added to the reaction mixture and stirring was continued overnight at room temperature. After evaporation, the residue was dissolved in $CH_2Cl_2$ (10–30 mL), washed successively with 5% aq citric acid, water, and 5% aq $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by column chromatography to give the corresponding derivatives **10** or **11**.

#### 5.4.1. (2*E*)-3-(Allyloxycarbonyl)-2-heptylidenepropionyl-(*S*)-2-phenyltryptophan-*N*-methylamide (10x)

Prepared according to the general procedure 5.4: acid **9v** (195 mg, 0.76 mmol); base: NMM (56 μL, 0.51 mmol); solvent: THF (5 mL); DMTMM (212 mg, 0.76 mmol); time of activation 0.5 h; (*S*)-2-phenyltryptophan-*N*-methylamide **8** (150 mg, 0.51 mmol); reaction time 18 h. After extraction, the crude product was purified by column chromatography (silica gel, $CH_2Cl_2$/MeOH, 99:1). Yield: **10x** (192 mg, 71%). Yellow amorphous solid. IR (film, cm$^{-1}$): 3295 (NH), 2916 (CH), 1731 (CO), 1652 (CO), 1502, 1454, 1361, 1308, 1172, 987, 921, 767; $^1$H NMR (300 MHz, $CDCl_3$): 0.88 (t, 3H, *J* = 6.5 Hz, –CH$_3$), 1.14–1.26 (m, 8H, CH$_2$), 1.95–2.02 (m, 2H, –C$H_2$–CH=), 2.41 (d, 3H, *J* = 4.7 Hz, CO–NH–C$H_3$), 3.15–3.50 (m, 4H, NH–CO–C$H_2$, CH–C$H_2$–indole), 4.46–4.48 (m, 2H, CH$_2$–O), 4.62–4.67 (m, 1H, C$H$–CH$_2$-indole), 5.13–5.25 (m, 2H, C$H_2$=CH–CH$_2$O), 5.76–5.95 (m, 3H, –C$H$=C, CH$_2$=C$H$–CH$_2$O, CO–N$H$–CH$_3$), 6.64 (d, 1H, *J* = 7.3 Hz, CO–NH), 7.03–7.75 (m, 9H, $H_{aryl}$, $H_{indole}$), 9.15 (s, 1H, $NH_{indole}$); $^{13}$C NMR (75 MHz, $CDCl_3$): 13.9, 22.4, 26.1, 28.1, 28.2, 28.9, 29.3, 31.5, 32.0, 54.3, 65.2, 107.2, 110.9, 118.9, 119.1, 119.7, 122.3, 127.9, 128.0, 128.7, 128.8, 131.6, 132.3, 135.8, 138.3, 168.1, 170.2, 171.3; MS (ESI, *m/z*, %): 530.3 (M+1, 100), 499 (14).

#### 5.4.2. (2*E*)-3-(Allyloxycarbonyl)-2-(3-phenylpropylidene)-propionyl-(*S*)-2-phenyltryptophan-*N*-methylamide (10y)

Prepared according to the general procedure 5.4: acid **9w** (162 mg, 0.59 mmol); base: NMM (62 μL, 0.56 mmol); solvent: THF (5 mL); DMTMM (233 mg, 0.84 mmol); time of activation 0.5 h; (*S*)-2-phenyltryptophan-*N*-methylamide **8** (165 mg, 0.56 mmol); reaction time 18 h. After extraction the crude product was purified by column chromatography (silica gel, $CH_2Cl_2$/MeOH, 98:2). Yield: **10y** (300 mg, 93%). Yellow amorphous solid. IR (film, cm$^{-1}$): 3303 (NH), 2916 (CH), 1727 (CO), 1652 (CO), 1599, 1533, 1454, 1366, 1304, 1176, 1132, 987, 930, 740; $^1$H NMR (300 MHz, $CDCl_3$): 2.32–2.37 (m, 2H, C$H_2$–CH$_2$–Ph), 2.51 (d, 3H, *J* = 4.8 Hz, CO–NH–C$H_3$), 2.57–2.63 (m, 2H, CH$_2$–C$H_2$–Ph), 3.12–3.51 (m, 4H, CO–C$H_2$, CH–C$H_2$-indole), 4.48–4.50 (m, 2H, CH$_2$–O), 4.69–4.71 (m, 1H, C$H$–CH$_2$-indole), 5.15–5.29 (m, 2H, C$H_2$=CH–CH$_2$O), 5.78–5.84 (m, 1H, CH$_2$=C$H$–CH$_2$O), 5.95 (t, 1H, *J* = 7.3 Hz, –CH=C), 6.57 (d, 1H, *J* = 7.3 Hz, NH), 7.09–7.57 (m, 14H, $H_{aryl}$, $H_{indole}$), 7.83 (d, 1H, *J* = 7.6 Hz, NH), 8.41 (s, 1H, $NH_{indole}$); $^{13}$C NMR (75 MHz, $CDCl_3$): 26.2, 27.2, 30.3, 32.5, 34.6, 55.8, 65.6, 107.8, 110.8, 118.4, 119.5, 120.1, 122.7, 126.1, 127.9, 128.0, 128.2, 128.4, 128.9, 129.1, 130.1, 131.6, 132.4, 135.7, 136.7, 140.7, 168.3, 170.7, 171.4; MS (ESI, *m/z*, %): 550 (M+1, 90), 519 (83), 287 (15), 274 (25), 257 (100), 239 (25), 218 (63), 206 (82).

#### 5.4.3. 3-(*R*)-[2-(*S*)-(2-Phenyl-1*H*-indol-3-yl)-methylcarbamoyl)-ethylcarbamoyl]-5-methyl *tert*-butylhexanoate (11z)

Prepared according to the general procedure 5.4: acid **7** (399 mg, 1.73 mmol); base: NMM (205 μL, 1.87 mmol); solvent: THF (20 mL); DMTMM (570 mg, 2.01 mmol); time of activation 0.5 h; (*S*)-2-phenyltryptophan-*N*-methylamide **8** (500 mg, 1.70 mmol); reaction time 3 h. After extraction, the crude product was purified by column chromatography (silica gel, $CH_2Cl_2$/MeOH, 98:2). Yield: **11z** (620 mg, 72%). Yellowish amorphous solid. $[\alpha]_D^{21}$ +21 (*c* 0.6, DMSO); IR (KBr, cm$^{-1}$): 3383 (NH), 3286 (NH), 2925 (CH), 1727 (CO), 1639 (CO), 1547, 1525, 1450, 1366, 1300, 1252, 1150, 838, 767; $^1$H NMR (300 MHz, DMSO-$d_6$): 0.79 (d, 3H, *J* = 6.2 Hz, CH(C$H_3$)$_2$), 0.85 (d, 3H, *J* = 6.3 Hz, CH(C$H_3$)$_2$), 1.07 (m, 1H, C$H_2$–CH(CH$_3$)$_2$), 1.38 (m, 11H, C(C$H_3$)$_3$, CH$_2$–C$H$(CH$_3$)$_2$, C$H_2$–CH(CH$_3$)$_2$), 2.10–2.27 (m, 2H, O–CO–C$H_2$), 2.38 (d, 3H, *J* = 4.6 Hz, CONHC$H_3$), 2.70 (m, 1H, C$H$–CH$_2$–CH(CH$_3$)$_2$), 2.99–3.32 (m, 2H, CH–C$H_2$-indole), 4.59 (m, 1H, C$H$–CH$_2$-indole), 7.01 (m, 1H, H6$_{indole}$), 7.07 (m, 1H, H5$_{indole}$), 7.37 (m, 2H, $H_{aryl}$), 7.50 (m, 2H, $H_{aryl}$, H7$_{indole}$), 7.62 (d, 1H, *J* = 4.6 Hz, CO–N$H$–CH$_3$), 7.67 (d, 1H, *J* = 7.8 Hz, H4$_{indole}$), 7.73 (m, 2H, $H_{aryl}$), 11.20 (s, 1H, $NH_{indole}$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): 22.6, 23.2, 25.4, 25.9, 27.9, 28.8, 38.2, 40.0, 41.3, 54.3, 79.9, 107.9, 111.2, 118.8, 119.5, 121.7, 127.5, 128.3, 128.9, 129.3, 133.0, 135.4, 136.1, 171.1, 171.7, 173.7; MS (CI, *m/z*, %): 506.28 (M+1, 100), 450 (29).

### 5.5. General procedure for the preparation of carboxylic acids 12v–y

To a solution of allyl ester **10v-y** in THF *tetrakis*(triphenylphosphine)palladium (Pd(PPh$_3$)$_4$) and morpholine were added and the reaction mixture was stirred for 30 min under nitrogen atmo-

sphere. After evaporation of the solvent, the residue was dissolved in $CH_2Cl_2$ (15 mL) and extracted with 5% aq $NaHCO_3$ (15 mL). The aqueous phase was acidified (pH 1) with 5% aq citric acid solution, extracted with $CH_2Cl_2$. The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated in vacuo to give acids **12v–y**.

#### 5.5.1. (2*E*)-3-(Hydroxycarbonyl)-2-heptylidenepropionyl-(*S*)-2-phenyltryptophan-*N*-methylamide (12x)

Prepared according to the general procedure 5.5: allyl ester **10x** (190 mg, 0.36 mmol); solvent: THF (5 mL); $Pd(PPh_3)_4$ (42 mg, 0.036 mmol); base: morpholine (98 μL, 1.11 mmol); Yield: **12x** (69 mg, 40%). Viscous oil. IR (film, $cm^{-1}$): 3295 (NH), 2916 (CH), 1709 (CO), 1647 (CO), 1528, 1458, 1304, 1264, 1181, 1009, 745; $^1H$ NMR (300 MHz, $CDCl_3$): 0.87 (t, 3H, $J$ = 6.3 Hz, $-CH_3$), 1.14–1.43 (m, 8H, $CH_2$), 2.02–2.09 (m, 2H, $-CH_2-CH=$), 2.37 (d, 3H, $J$ = 4.4 Hz, CO–NH–$CH_3$), 3.08–3.46 (m, 4H, NH–CO–$CH_2$, CH–$CH_2$-indole), 4.62–4.69 (m, 1H, $CH$–$CH_2$-indole), 5.86 (q, 1H, $J$ = 4.4 Hz, CO–N$H$–$CH_3$), 6.04 (t, 1H, $J$ = 7.3 Hz, CH=), 7.05–7.69 (m, 10H, NH, $H_{aryl}$, $H_{indole}$), 8.80 (s, 1H, $NH_{indole}$), 10.50 (br s, 1H, OH); $^{13}C$ NMR (75 MHz, $CDCl_3$): 13.9, 22.4, 26.2, 27.6, 28.3, 28.4, 28.8, 31.4, 34.2, 54.1, 106.8, 111.0, 118.9, 119.9, 122.5, 127.5, 127.8, 128.4, 128.6, 128.7, 128.9, 131.8, 132.4, 135.7, 135.9, 140.4, 170.4, 171.4, 172.7; MS (ESI, *m/z*, %) 490 (M+1, 56), 459 (15), 279 (30), 79 (100).

#### 5.5.2. (2*E*)-3-(Hydroxycarbonyl)-2-(3-phenylpropylidene)-propionyl-(*S*)-2-phenyltryptophan-*N*-methylamide (12y)

Prepared according to the general procedure 5.5: allyl ester **10y** (281 mg, 0.51 mmol); solvent: THF (7 mL); $Pd(PPh_3)_4$ (59 mg, 0.051 mmol); base: morpholine (138 μL, 1.58 mmol); Yield: **12y** (169 mg, 65%). Viscous oil. IR (film, $cm^{-1}$): 3312 (OH), 3048 (NH), 2925 (CH), 1713 (CO), 1643 (CO), 1528, 1454, 1260, 1185, 908, 740; $^1H$ NMR (300 MHz, $CDCl_3$): 2.41–2.50 (m, 5H, $CH_2$–$CH_2$–Ph, CO–NH–$CH_3$), 2.57–2.67 (m, 2H, $CH_2$–$CH_2$–Ph), 3.10–3.50 (m, 4H, CO–$CH_2$, CH–$CH_2$-indole), 4.63–4.70 (m, 1H, $CH$–$CH_2$–indole), 5.44 (q, 1H, $J$ = 4.8 Hz, CO–N$H$–$CH_3$), 6.11 (t, 1H, $J$ = 7.3 Hz, CH=), 7.03–7.54 (m, 14H, $H_{aryl}$, $H_{indole}$), 7.77 (d, 1H, $J$ = 7.1 Hz, NH), 8.35 (s, 1H, $NH_{indole}$); $^{13}C$ NMR (75 MHz, $CDCl_3$): 26.3, 27.9, 30.2, 34.5, 34.6, 54.1, 107.1, 111.0, 119.1, 120.2, 122.7, 126.2, 127.9, 128.1, 128.2, 128.5, 128.7, 128.8, 129.1, 132.1, 132.3, 135.7, 135.9, 139.1, 140.4, 170.5, 171.1, 171.8; MS (ESI, *m/z*, %) 510 (M+1, 100), 479 (40).

#### 5.5.3. 3-(*R*)-[2-(*S*)-(2-Phenyl-1*H*-indol-3-yl)-methylcarbamoyl)-ethylcarbamoyl]-5-methyl hexanoic acid (12z)

To a solution of ester **11z** (570 mg, 1.13 mmol) in $CH_2Cl_2$ (11 mL), were added TFA (2.8 mL) and dithiothreitol (10 mg), and the mixture was stirred at room temperature for 3.5 h. After evaporation, a white solid was precipitated in ether. Yield: **12z** (325 mg, 64%). White crystals. Mp 197–198 °C; $[\alpha]_D^{21}$ +36.5 (*c* 1.17, DMSO); IR (KBr, $cm^{-1}$): 3568 (OH), 3286 (NH), 3057 (NH), 2952 (CH), 1701 (CO), 1635 (CO), 1538, 1454, 1247, 917, 763; $^1H$ NMR (300 MHz, DMSO-$d_6$): 0.78 (d, 3H, $J$ = 5.8 Hz, $CH(CH_3)_2$), 0.85 (d, 3H, $J$ = 5.8 Hz, $CH(CH_3)_2$), 1.11 (m, 1H, $CH_2$–$CH(CH_3)_2$), 1.37 (m, 2H, $CH_2$–$CH(CH_3)_2$, $CH_2$–$CH(CH_3)_2$), 2.23 (m, 2H, O–CO–$CH_2$), 2.39 (d, 3H, $J$ = 4.1 Hz, CO–NH–$CH_3$), 2.71 (m, 1H, $CH$–$CH_2$–$CH(CH_3)_2$), 3.18 (m, 2H, CH–$CH_2$-indole), 4.59 (m, 1H, $CH$–$CH_2$–indole), 7.00 (m, 1H, $H6_{indole}$), 7.10 (m, 1H, $H5_{indole}$), 7.37 (m, 2H, $H_{aryl}$), 7.50 (m, 2H, $H_{aryl}$, $H7_{indole}$), 7.61 (d, 1H, $J$ = 4.1 Hz, CO–N$H$–$CH_3$), 7.68 (d, 1H, $J$ = 7.8 Hz, $H4_{indole}$), 7.74 (m, 2H, $H_{aryl}$), 8.05 (d, 1H, $J$ = 8.0 Hz, CO–NH), 11.20 (s, 1H, $NH_{indole}$), 12.14 (m, 1H, OH); $^{13}C$ NMR (75 MHz, DMSO-$d_6$): 22.5, 23.1, 25.4, 25.9, 28.6, 37.1, 39.9, 41.3, 54.3, 108.0, 111.2, 118.8, 119.5, 121.7, 127.5, 128.2, 128.8, 129.3, 132.9, 135.3, 136.1, 171.6, 173.4, 174.0; MS (CI, *m/z*, %) 450.26 (M+1, 100), 419 (25), 218 (15).

### 5.6. General procedure for condensation of carboxylic acids 12v,w,x,z and 4-(4-bromophenyl)-benzenesulfonylhydrazide HCl salt

A mixture of acid **12** *N*-methylmorpholine (NMM), and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride) (DMTMM) in dry THF was stirred at room temperature for 30 min. 4-(4-Bromophenyl)benzenesulfonylhydrazide HCl salt in THF with NMM were added to the mixture. The reaction mixture was stirred overnight at room temperature. The organic phase was washed successively with 5% aq citric acid, water, 5% aq $NaHCO_3$ and dried over $MgSO_4$. The organic phase was filtered, evaporated in vacuo and residue was purified by column chromatography to give **4**.

#### 5.6.1. *N′*-{3-(*E*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-decenoyl}-*N*-4-(4-bromophenyl)benzenesulfonylhydrazide (4v)

Prepared according to the general procedure 5.6: acid **12v** (50 mg, 0.12 mmol); base: NMM (20 μL, 0.18 mmol); solvent: THF (3 mL); DMTMM (50 mg, 0.18 mmol); time of activation 0.5 h; 4-(4-bromophenyl)benzenesulfonylhydrazide HCl salt (132 mg, 0.36 mmol); solvent: THF (3 mL); base: NMM (40 μL, 0.36 mmol); reaction time 6 h. After extraction the crude product was purified by column chromatography (silica gel, $CH_2Cl_2$/MeOH, 98:2 then 95:5). Yield: **4v** (23 mg, 26%). Amorphous solid. IR (film, $cm^{-1}$): 3295 (NH), 2916 (CH), 1647 (CO), 1537, 1339 ($SO_2$), 1167 ($SO_2$), 1093, 811, 771, 745; $^1H$ NMR (300 MHz, $CDCl_3$): 0.83 (t, 3H, $J$ = 6.6 Hz, $-CH_3$), 1.11–1.25 (m, 8H, $CH_2$), 1.84–1.96 (m, 2H, $-CH_2-CH=$), 2.66 (d, 3H, $J$ = 4.6 Hz, CO–NH–$CH_3$), 3.02–3.34 (m, 4H, NH–CO–$CH_2$, CH–$CH_2$-indole), 4.71–4.73 (m, 1H, $CH$–$CH_2$-indole), 5.93 (q, 1H, $J$ = 4.6 Hz, CO–N$H$–$CH_3$), 6.07 (t, 1H, $J$ = 7.0 Hz, CH=), 6.70 (d, 1H, $J$ = 7.7 Hz, CO–NH), 7.09–7.86 (m, 14H, NH, $H_{aryl}$, $H_{indole}$), 8.35 (s, 1H, $NH_{indole}$), 9.51 (d, 1H, $J$ = 5.6 Hz, NH); $^{13}C$ NMR (75 MHz, $CDCl_3$): 14.0, 22.4, 26.3, 28.4, 28.6, 29.0, 31.5, 33.3, 54.4, 110.4, 111.4, 118.5, 119.8, 122.3, 123.1, 127.0, 127.3, 128.1, 128.7, 129.1, 132.1, 135.5, 136.0, 137.7, 140.6, 144.9, 168.9, 169.3, 171.7; HRMS Calcd for $C_{35}H_{40}N_5O_5NaSBr$: 744.1831. Found: 744.1843; Anal. Calcd for $C_{35}H_{40}N_5O_5SBr{\cdot}H_2O$: C 56.81, H 5.41, N 9.46. Found: C 56.25, H 5.77, N 9.40.

#### 5.6.2. *N′*-{3-(*E*)-[2-(*S*)-(1*H*-Indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-6-phenylhexenoyl}-*N*-4-(4-bromophenyl)-benzenesulfonylhydrazide (4w)

Prepared according to the general procedure 5.6: acid **12w** (75 mg, 0.17 mmol); base: NMM (28 μL, 0.26 mmol); solvent: THF (5 mL); DMTMM (72 mg, 0.26 mmol); time of activation 0.5 h; 4-(4-bromophenyl)benzenesulfonylhydrazide HCl salt (157 mg, 0.43 mmol); solvent: THF (5 mL); base: NMM (48 μL, 0.43 mmol); reaction time 18 h. After extraction the crude product was purified by column chromatography (silica gel, $CH_2Cl_2$/MeOH, 98:2 then 95:5). Yield: **4w** (33 mg, 26%). Amorphous solid. IR (film, $cm^{-1}$): 3330 (NH), 2916 (CH), 1727 (CO), 1458, 1374 ($SO_2$), 1260, 1163 ($SO_2$), 1093, 1018, 798, 745; $^1H$ NMR (300 MHz, $CDCl_3$): 2.18–2.25 (m, 2H, $CH_2$–$CH_2$–Ph), 2.46 (t, 2H, $J$ = 7.6 Hz, $CH_2$–$CH_2$–Ph), 2.65 (d, 3H, $J$ = 4.8 Hz, CO–NH–$CH_3$), 2.97 (s, 2H, NH–CO–$CH_2$), 3.11–3.32 (m, 2H, CH–$CH_2$-indole), 4.69–4.74 (m, 1H, $CH$–$CH_2$-indole), 5.97 (q, 1H, $J$ = 4.8 Hz, CO–N$H$–$CH_3$), 6.09 (t, 1H, $J$ = 7.1 Hz, CH=), 6.81 (d, 1H, $J$ = 7.6 Hz, CO–NH), 6.99–8.31 (m, 19H, NH, $H_{aryl}$, $H_{indole}$), 8.31 (s, 1H, $NH_{indole}$), 9.45 (d, 1H, $J$ = 4.6 Hz, NH); $^{13}C$ NMR (75 MHz, $CDCl_3$): 26.3, 28.3, 30.3, 33.2, 34.3, 54.3, 110.4, 111.4, 118.5, 119.7, 122.3, 123.0, 126.2, 127.0, 127.3, 128.1, 128.3, 128.5, 128.7, 128.8, 128.9, 129.0, 132.1, 132.1, 135.5, 136.1, 137.7, 139.0, 140.3, 144.9, 168.8, 169.2, 171.6; HRMS Calcd for $C_{37}H_{36}N_5O_5NaSBr$: 764.1518. Found: 764.1522.

### 5.6.3. *N′*-{3-(*E*)-[2-(*S*)-(2-Phenyl-1*H*-indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-decenoyl}-*N*-4-(4-bromophenyl)-benzenesulfonylhydrazide (4x)

Prepared according to the general procedure 5.6: acid **12x** (50 mg, 0.1 mmol); base: NMM (17 μL, 0.15 mmol); solvent: THF (3 mL); DMTMM (42 mg, 0.15 mmol); time of activation 0.5 h; 4-(4-bromophenyl)benzenesulfonylhydrazide HCl salt (111 mg, 0.3 mmol); solvent: THF (3 mL); base: NMM (34 μL, 0.3 mmol); reaction time 7 h. After extraction the crude product was purified by column chromatography (silica gel, $CH_2Cl_2$/MeOH, 99:1 then 98:2). Yield: **4x** (24 mg, 30%). Amorphous solid. IR (film, $cm^{-1}$): 3259 (NH), 2925 (CH), 1661 (CO), 1647 (CO), 1506, 1344 ($SO_2$), 1167 ($SO_2$), 1115, 1093, 811, 771, 740; $^1H$ NMR (300 MHz, $CDCl_3$): 0.84 (t, 3H, $J$ = 6.6 Hz, –$CH_3$), 1.12–1.25 (m, 8H, $CH_2$), 1.77–1.93 (m, 2H, –$CH_2$–CH═), 2.49 (d, 3H, $J$ = 4.7 Hz, CO–NH–$CH_3$), 2.96 (s, 2H, NH–CO–$CH_2$), 3.29–3.47 (m, 2H, CH–$CH_2$–indole), 4.66–4.70 (m, 1H, C$H$–$CH_2$-indole), 5.53 (q, 1H, $J$ = 4.7 Hz, CO–N$H$–$CH_3$), 5.93 (t, 1H, $J$ = 7.1 Hz, CH═), 6.78 (d, 1H, $J$ = 7.3 Hz, CO–NH), 7.26–7.87 (m, 18H, NH, $H_{aryl}$, $H_{indole}$), 8.44 (s, 1H, $NH_{indole}$), 9.62 (d, 1H, $J$ = 5.8 Hz, NH); $^{13}C$ NMR (75 MHz, $CDCl_3$): 14.0, 22.4, 26.3, 28.2, 28.4, 28.6, 29.0, 31.5, 33.5, 54.0, 107.3, 111.0, 119.1, 120.3, 122.7, 123.0, 127.0, 127.9, 128.0, 128.1, 128.4, 128.5, 128.7, 128.9, 129.1, 131.9, 132.1, 132.4, 135.5, 135.7, 136.0, 137.8, 140.2, 144.8, 168.8, 169.2, 171.2; HRMS Calcd for $C_{41}H_{44}N_5O_5NaSBr$: 820.2127. Found: 820.2144.

### 5.7. *N*-{3-(*E*)-[2-(*S*)-(2-Phenyl-1*H*-indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-6-phenylhexenoyl}-*N*-*tert*-butoxycarbonyl-hydrazide (13y)

A mixture of acid **12y** (160 mg, 0.3 mmol), *N*-methylmorpholine (NMM) (35 μL, 0.3 mmol) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride) (DMTMM) (130 mg, 0.4 mmol) in dry THF (7 mL) was stirred at room temperature for 30 min. Boc-hydrazine (46 mg, 0.3 mmol) was added to the precedent mixture and stirring was continued for 1.5 h. After evaporation of the solvent, the residue was dissolved in $CH_2Cl_2$ (10 mL) and washed successively with 5% aq citric acid solution, water and 5% aq $NaHCO_3$ solution. The organic layer was dried ($MgSO_4$), filtered and the filtrate was evaporated in vacuo. The crude product was purified by column chromatography (silica gel, elution: $CH_2Cl_2$/MeOH, 98:2 then 96:4) to give **13y** as a pale-yellow foam. Yield: **13y** (176.6 mg, 92%). $[\alpha]_D^{21}$ −20.2 (*c* 0.5, $CHCl_3$); IR (film, $cm^{-1}$): 3295 (NH), 2925 (CH), 1735 (CO), 1652 (CO), 1511, 1458, 1370, 1238, 1159, 740; $^1H$ NMR (300 MHz, $CDCl_3$): 1.43 (s, 9H, C($CH_3$)$_3$), 2.39–2.63 (m, 7H, C$H_2$–$CH_2$–Ph, CO–NH–$CH_3$), 2.97–3.11 (m, 2H, CO–$CH_2$), 3.41–3.49 (m, 2H, CH–$CH_2$–indole), 4.64–4.67 (m, 1H, C$H$–$CH_2$-indole), 5.83 (br s, 1H, NH), 5.91 (t, 1H, $J$ = 6.6 Hz, CH═), 6.63 (d, 1H, $J$ = 6.9 Hz, NH), 7.12–7.56 (m, 14H, $H_{aryl}$, $H_{indole}$), 7.76 (d, 1H, $J$ = 7.7 Hz, NH), 8.42 (s, 1H, $NH_{indole}$), 8.53 (s, 1H, NH); $^{13}C$ NMR (75 MHz, $CDCl_3$): 26.3, 27.5, 28.0, 30.5, 33.5, 34.5, 54.1, 81.4, 107.5, 110.9, 119.3, 120.2, 122.7, 126.1, 127.9, 128.0, 128.3, 128.4, 128.9, 129.1, 129.6, 132.4, 135.7, 138.2, 140.8, 155.2, 169.4, 169.7, 171.3; MS (ESI, *m*/*z*, %) 624 (M+1, 100), 568 (48), 537 (20), 524 (15), 492 (40).

### 5.8. *N′*-{3-(*E*)-[2-(*S*)-(2-Phenyl-1*H*-indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-6-phenylhexenoyl}-hydrazide (14y)

A solution of Boc protected hydrazide **13y** (166 mg, 0.26 mmol) in methanol saturated with HCl gas (1.4 mL, C 11.3 mol/L) was stirred at room temperature for 45 min. After evaporation of the solvent, the crude residue was dissolved in $CH_2Cl_2$ and made alkaline (pH 9) at 0 °C by addition of an 1 N aq NaOH solution. The precipitate was extracted with $CH_2Cl_2$ (3× 5 mL), dried ($MgSO_4$), filtered and evaporated. Yield: **14y** (78 mg, 55%). Amorphous solid. IR (film, $cm^{-1}$): 3277 (NH), 2916 (CH), 1647 (CO), 1612 (CO), 1528, 1445, 1405, 1150, 740; $^1H$ NMR (300 MHz, $CD_3OD$): 2.23–2.31 (m, 2H, C$H_2$–$CH_2$–Ph), 2.41–2.56 (m, 5H, $CH_2$–C$H_2$–Ph, CO–NH–C$H_3$), 2.76–2.96 (m, 2H, CO–C$H_2$), 3.34–3.49 (m, 2H, CH–C$H_2$–indole), 4.51–4.55 (m, 1H, C$H$–$CH_2$-indole), 5.86 (t, 1H, $J$ = 7.3 Hz, CH═), 6.98–7.59 (m, 14H, $H_{aryl}$, $H_{indole}$); $^{13}C$ NMR (75 MHz, $CD_3OD$): 25.4, 26.7, 29.9, 31.8, 34.0, 54.0, 106.0, 110.7, 118.2, 118.9, 121.6, 125.6, 127.2, 127.6, 127.8, 127.9, 128.3, 128.4, 128.6, 129.2, 132.5, 135.7, 137.9, 140.4, 169.1, 170.5, 172.1; MS (ESI, *m*/*z*, %) 524 (M+1, 100), 492 (15).

### 5.9. *N′*-{3-(*E*)-[2-(*S*)-(2-Phenyl-1*H*-indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-6-phenylhexenoyl}-*N*-4-(4-bromophenyl)benzenesulfonylhydrazide (4y)

Prepared according to the general procedure 5.2: hydrazide **14y** (52 mg, 0.099 mmol); solvent: THF (5 mL); base: triethylamine (14 μL, 0.099 mmol); 4-(4-bromophenyl) benzenesulfonylchloride (36 mg, 0.1 mmol); reaction time 18 h. After extraction, the crude product was purified by column chromatography (silica gel, $CH_2Cl_2$/MeOH, 98:2). Yield: **4y** (44 mg, 54%). Amorphous solid. IR (film, $cm^{-1}$): 3392 (NH), 3277 (NH), 2916 (CH), 1661 (CO), 1643 (CO), 1511, 1454, 1339 ($SO_2$), 1255, 1159 ($SO_2$), 1093, 811, 740; $^1H$ NMR (300 MHz, $CDCl_3$): 2.15–2.26 (m, 2H, C$H_2$–$CH_2$–Ph), 2.43–2.55 (m, 5H, $CH_2$–C$H_2$–Ph, CO–NH–C$H_3$), 2.91–2.97 (m, 2H, NH–CO–C$H_2$), 3.29–3.46 (m, 2H, CH–C$H_2$-indole), 4.61–4.69 (m, 1H, C$H$–$CH_2$-indole), 5.51 (q, 1H, $J$ = 4.6 Hz, CO–N$H$–$CH_3$), 5.95 (t, 1H, $J$ = 7.2 Hz, CH═), 6.75 (d, 1H, $J$ = 7.4 Hz, CO–NH), 6.99–7.86 (m, 23H, NH, $H_{aryl}$, $H_{indole}$), 8.33 (s, 1H, $NH_{indole}$), 9.52 (d, 1H, $J$ = 7.4 Hz, NH); $^{13}C$ NMR (75 MHz, $CDCl_3$): 26.3, 28.2, 30.4, 33.4, 34.4, 54.0, 107.3, 111.1, 119.1, 120.3, 122.7, 122.9, 126.2, 127.0, 127.9, 128.1, 128.5, 128.7, 128.9, 128.9, 129.1, 129.1, 132.0, 132.4, 135.6, 135.7, 135.9, 137.7, 138.7, 140.4, 144.8, 168.7, 169.1, 171.2; HRMS Calcd for $C_{43}H_{40}N_5O_5NaSBr$: 840.1846. Found: 840.1831.

### 5.10. *N′*-{3-(*R*)-[2-(*S*)-(2-Phenyl-1*H*-indol-3-yl)-1-(methylcarbamoyl)-ethylcarbamoyl]-5-methylhexanoyl}-*N*-4-(4-bromophenyl)benzenesulfonylhydrazide (4z)

Prepared according to the general procedure 5.6: acid **12z** (42 mg, 0.09 mmol); base: NMM (25 μL, 0.13 mmol); solvent: THF (3 mL); DMTMM (38 mg, 0.14 mmol); time of activation 0.5 h; 4-(4-bromophenyl)benzenesulfonylhydrazide HCl salt (84 mg, 0.23 mmol); solvent: THF (3 mL); base: NMM (15 μL, 0.14 mmol); reaction time 5 h. After extraction, the crude product was purified by column chromatography (silica gel, $CH_2Cl_2$/MeOH, 99:1 then 98:2). Yield: **4z** (24 mg, 35%). Amorphous solid. $[\alpha]_D^{21}$ +8 (*c* 0.25, MeOH); IR (film, $cm^{-1}$): 3256 (NH), 3057 (NH), 2919 (CH), 1633 (CO), 1508, 1340 ($SO_2$), 1161 ($SO_2$) 1094, 806, 700; $^1H$ NMR (300 MHz, $CD_3OD$): 0.68–0.74 (m, 6H, CH($CH_3$)$_2$), 0.79 (m, 1H, $CH_2$–C$H$($CH_3$)$_2$), 1.25–1.28 (m, 2H, C$H_2$–CH($CH_3$)$_2$), 2.06–2.10 (m, 2H, NH–CO–C$H_2$), 2.38 (s, 3H, CO–NH–C$H_3$), 2.42–2.50 (m, 1H, C$H$–$CH_2$–CH($CH_3$)$_2$), 3.16–3.18 (m, 2H, CH–C$H_2$–indole), 4.52–4.53 (m, 1H, C$H$–$CH_2$-indole), 6.99–7.92 (m, 17H, $H_{aryl}$, $H_{indole}$); $^{13}C$ NMR (75 MHz, $CDCl_3$): 22.4, 23.3, 26.4, 26.7, 28.9, 37.1, 42.0, 42.2, 55.7, 108.2, 112.0, 120.0, 120.1, 122.9, 128.2, 128.5, 129.3, 129.9, 130.0, 130.6, 133.2, 133.3, 134.5, 137.1, 137.7, 168.4, 173.9, 176.6; HRMS Calcd for $C_{38}H_{40}N_5O_5NaSBr$: 780.1819. Found: 780.1831.

### 5.11. Materials and methods for biological evaluation

Quenched fluorogenic substrates DNP-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(*N*-Me-Abz)-$NH_2$ [where DNP: 2,4-dinitrophenyl; Cha:

β-cyclohexylalanyl; Abz: 2-aminobenzoyl(anthraniloyl)] were purchased from Calbiochem (VWR, Strasbourg, France). Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-$NH_2$ (where Mca: (7-methoxycoumarin-4-yl); Dpa: [*N*-3-(2,4-dinitrophenyl)-L-2,3-diaminopropionyl] and NBD-ε-aminocaproyl-Arg-Pro-Lys-Pro-Leu-Ala-Nva-Trp-Lys(DMAC-A)-$NH_2$ (where NBD: 6-(7-nitro-benzo[1,2,5]oxodiazol-4-yl); DMACA: 7-dimethylaminocoumarin-4-yl) were obtained from Bachem (Weil am Rhein, Germany). Human recombinant pro-MMP-2, pro-MMP-9, pro-MMP-1, pro-MMP-7, and catalytic domains of MT1-MMP or of MMP-3 were obtained from Calbiochem. The pro-enzymes were freshly activated with 1 μM (pro-MMP-2) or 2 μM (pro-MMP-9, pro-MMP-7, and pro-MMP-1) *p*-aminophenylmercuric acetate (Sigma–Aldrich, Saint Quentin Fallavier, France) at 37 °C in water bath for 2 h.

Enzyme inhibitions were carried out in 50 mM Tris–HCl buffer (pH 7.5), 150 mM NaCl, 5 mM $CaCl_2$ at 25 °C except for MMP-3 where 50 mM Tris–HCl (pH 7.5), 5 mM $CaCl_2$, 1 mM $ZnCl_2$ was instead used, according to manufacturer instructions. All assays were performed in black 96-well plates (non-binding surface plates 3650; CorningCostar), in conditions of initial rate of substrate hydrolysis. Progress curves were monitored by following the increase of fluorescence at (i) 465 nm ($\lambda_{ex}$ = 326 nm) induced by the cleavage of MCA-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-$NH_2$ (Bachem) by MMP-2 or MMP-7; (ii) 465 nm ($\lambda_{ex}$ = 360 nm) induced by the cleavage of Dup-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys (*N*-MeAbz)-$NH_2$ (Calbiochem) by either MMP-1 or MMP-9; (iii) 465 nm ($\lambda_{ex}$ = 360 nm) induced by the cleavage of NBD-ε-aminocaproyl-Arg-Pro-Lys-Pro-Leu-Ala-Nva-Trp-Lys(DMACA)-$NH_2$ by MMP-3. The fluorescence was monitored with a Perkin-Elmer HT Soft 7000 Plus spectrofluorimeter (Perkin-Elmer, Courtaboeuf, France) equipped with a temperature device control and a plate shaker.

Typical experimental conditions were 200 μL of buffer, 1–2 nM of MMP and 1–10 μM of substrate. Inhibitors at various concentrations were preincubated for 30 min at 25 °C with enzymes before additions of substrate. Percentage inhibition was determined in triplicate experiments at 5–7 concentrations selected to observe a 20–80% range of inhibition. $IC_{50}$ values were determined by fitting the $v_i/v_o$ versus inhibitor concentration curve using a nonlinear regression program (Grafit Computer program, R. Leatherbarrow, Erithacus Software).

### 5.12. Molecular modelling studies

#### 5.12.1. MMPs and ligands initial data

Structures of Protein Data Bank (PDB) entries 1CK7 for MMP-2[28] and 1GKC for MMP-9[29] were used for docking simulations. While MMP atoms and the zinc ion in its catalytic site were retained, all the other atoms were removed. The positions of polar hydrogens and charges were assigned using the Kollman algorithm.[30] Solvation parameters were added using the ADDSOL utility of AutoDock 4.0. MMP side chains were kept fixed for all the docking computations.

Ligands were built using the module BIOPOLYMER of Insight98 software package (MSI, Inc., San Diego, CA, USA). Ligands structure was optimized and their atoms charges were computed from quantum calculations with the Gaussian 03 program,[31] using a 6-31G* basis set. The AutoDock module AutoTors was used to define ligands torsion angles. All the flexible torsions, except the amide bonds, were allowed to rotate during the docking stage.

#### 5.12.2. Molecular docking simulations and calculations

Affinity grid maps were calculated for each atom type constituting the MMPs with AutoGrid program. Grid maps were centered on the MMP catalytic site, with 124 × 124 × 124 grid points and a spacing of 0.214 Å between the grid points. The distance-dependent dielectric permittivity of Mehler and Solmajer[32] was used for the calculations of the electrostatic grid maps. Random starting positions on the entire protein surface, random orientations and torsions were used for all ligands. The AutoDock program, version 4.0 was used for docking computations, with a Lamarckian genetic algorithm.[23,24] Each docking experiment was performed with two runs constituted of a series of 250 simulations. Each docking simulation was carried out with an initial population of 250 individuals, a maximum number of 5,000,000 energy evaluations and a maximum number of 50,000 generations. The pseudo-Solis and Wets modification methods were used with default parameters. Docked conformations of the ligands were clustered with a root mean square deviation (RMSD) cut-off of 0.5 Å. The molecular graphics of the three-dimensional structures and their display have been done using PyMOL program (Warren L. DeLano 'The PyMOL Molecular Graphics System.' DeLano Scientific LLC, San Carlos, CA, USA. http://www.pymol.org).

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