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Synthesis and carbonic anhydrase inhibitory properties of novel 4-(2-aminoethyl)benzenesulfonamide-dipeptide conjugates[☆]

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ABSTRACT

Thirty novel sulfonamide derivatives incorporating dipeptide were synthesized by facile acylation through benzotriazole mediated reactions and their structures were identified by ^{1}H NMR, ^{13}C NMR, MS and FT-IR spectroscopic techniques and elemental analysis. The carbonic anhydrase (CA, EC 4.2.1.1) inhibitory activity of the new compounds was assessed against four human (h) isoforms, hCA I, hCA II, hCA IV and hCA XII. Most of the synthesized compounds showed excellent *in vitro* carbonic anhydrase inhibitory properties comparable to those of the clinically used drug acetazolamide (AAZ). The new unprotected dipeptide-sulfonamide conjugates showed very effective inhibitory activity, in the low nanomolar range against II and XII, being less effective as hCA I and IV inhibitors. Four of the thirty compounds also showed strong inhibitory activity against hCA XII compared to AAZ.

1. Introduction

Sulfonamides were the first successfully synthesized antimicrobial drugs, with sulfanilamide as the prototype of “sulfa drugs”. The antimicrobial action mechanism of such sulfonamides involves competitive inhibition of folic acid synthesis which prevents the proliferation of the bacteria [1]. Sulfonamides are compounds with a broad spectrum of biological applications and can act as potential medicinal molecules in drug discovery and drug development [2–5]. They are particularly effective for the carbonic anhydrase enzyme inhibition and it is known that there are many connections between carbonic anhydrase and cancer [6–9]. Some primary sulfonamide derivatives (with the general formula $ArSO_2NH_2$) are in clinical use for treatment of glaucoma, epilepsy, obesity and cancer in connection with their carbonic anhydrase inhibition properties [10]. On the other hand, sulfa drugs (possessing the general formula $ArSO_2NHR$, i.e., secondary sulfonamides where Ar is 4-aminophenyl and R comprise a variety of diverse moieties) are among the first antibacterials which were and are still successfully used for fighting many common infections, although serious drug resistance problems emerged [6]. Although many sulfonamide agents are clinically used drugs, synthesizing their new derivatives with less side effect and improved selectivity is still of great interest in pharmaceutical chemistry. Due to bacterial resistance against sulfonamides and other classes of antibacterials, as well as many side effects, it is important to find efficient methods for the synthesis of new scaffolds incorporating the sulfonamide motif. In recent decades, morbidity and mortality associated with drug-resistant bacteria has increased alarmingly, currently accounting for hundreds of thousands of hospitalizations and tens of thousands of deaths annually in the worldwide [11]. On the other hand, it is known that short peptide sequences present a broad spectrum of biological activity, whereas low molecular weight peptides can also easily cross biological barriers, being less susceptible to protease attacks. In this respect, the synthesis of biologically important active molecules which mimic biological systems, obtained by reacting various dipeptides with sulfonamide derivatives are especially important to generate active sulfonamide derivatives which might overcome bacterial drug resistance and easily cross biological barriers. Furthermore, dipeptides have very interesting functions such as controlling cellular pH [12], antioxidation [13] and sweetener [14] and hence, we focused on to synthesize dipeptide-sulfonamide conjugates.

[☆] This work is part of PhD thesis experiments of N. Buğday.

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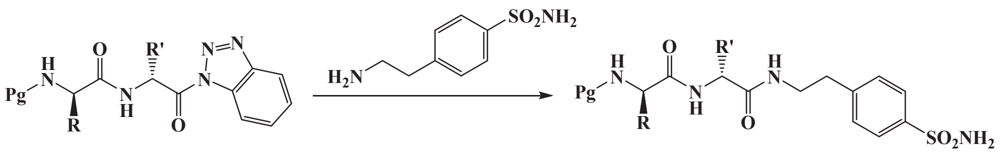


1) Pg= Z, R= H
R'= H
2) Pg= Z, R= H
R'= $CH_2CH_2SCH_3$
3) Pg= Z, R= CH_3
R'= H
4) Pg= Z, R= CH_3
R'= CH_3
5) Pg= Z, R= CH_3
R'= CH_2Ph
6) Pg= Z, R= CH_3
R'= CH_2SCH_2Ph
7) Pg= Z, R= CH_3
R'= $CH(CH_3)_2$
8) Pg= Z, R= CH_3
R'= $CH_2CH_2SCH_3$
9) Pg= Z, R= CH_2Ph
R'= H
10) Pg= Z, R= CH_2Ph
R'=CH_3
11) Pg= Z, R= CH_2Ph
R'= CH_2Ph
12) Pg= Z, R= CH_2Ph
R'= CH_2SCH_2Ph
13) Pg= Z, R= CH_2Ph
R'= $CH_2CH(CH_3)_2$
14) Pg= Z, R= CH_2Ph
R'= $CH(CH_3)CH_2CH_3$
15) Pg= Z, R= CH_2Ph
R'= $CH(CH_3)_2$
16) Pg= Z, R= CH_2Ph
R'= $CH_2CH_2SCH_3$
17) Pg= Z, R= $CH_2CH(CH_3)_2$
R'= CH_2Ph
18) Pg= Z, R= $CH_2CH_2SCH_3$
R'= H
19) Pg= Z, R= $CH_2CH_2SCH_3$
R'= CH_3
20) Pg= Z, R= $CH_2CH_2SCH_3$
R'= CH_2Ph
21) Pg= Z, R= $CH_2CH_2SCH_3$
R'= $CH_2CH_2SCH_3$
22) Pg= Boc, R= CH_2Ph
R'= H
23) Pg= Boc, R= CH_2Ph
R'= CH_2Ph
24) Pg= Boc, R= CH_2Ph
R'= $CH_2CH(CH_3)_2$
25) Pg= Boc, R= CH_2Ph
R'= $CH(CH_3)CH_2CH_3$
26) Pg= Boc, R= CH_2Ph
R'= $CH_2CH_2SCH_3$

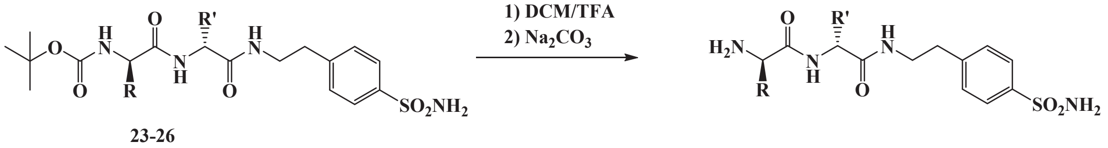
Scheme 1. Synthetic pathways of *N*-protected peptide-benzimidazole conjugates, 1–26.

From these perspectives, following our synthetic and carbonic anhydrase (CA, EC 4.2.1.1) screening work on *N*-protected amino acid-sulfonamide and dipeptide-homosulfonamide conjugates [15,16], we now report on inhibition capacities of newly synthesized *N*-protected and unprotected dipeptide-sulfonamide conjugates acting as inhibitors of physiologically relevant human (h) CA isoforms, such as hCA I, II, IV and XII enzymes.

2. Results and discussion

2.1. Synthesis and characterization of the new dipeptide-sulfonamides derivatives

The syntheses of novel 4-aminoethylsulfonamide-dipeptide conjugates reported in this study are depicted in Schemes 1 and 2. Because N-(protected-α-aminoacyl)benzotriazoles have enabled easy preparation of biologically important peptides and their several conjugates in high yield and purity under mild reaction conditions, with full retention of the original chirality [17–23], we chose benzotriazole-mediated methodology to synthesize the targeted dipeptide-sulfonamide conjugates. Compounds **1**–**26** were prepared through a facile benzotriazole mediated acylation reaction in one step (Scheme 1) at 70 °C under microwave irradiation for 30 min in dry THF with good or high yields and were purified by crystallization from ethanol. In order to learn the effect of the protection group, we transformed *N*-protected dipeptide-sulfonamide conjugates to unprotected dipeptide-sulfonamide conjugates (Scheme 2). Unprotected dipeptide-sulfonamide conjugates, **27**, **28**, **29** and **30** were obtained from the reaction of compounds **26**, **25**, **24** and **23** with TFA at room temperature for 1 h, respectively. All the compounds were fully characterized by ^1H, ^{13}C NMR, MS and FTIR (ATR) spectroscopy and elemental analyses. All spectral data were in agreement with the proposed structures. The characteristic NH resonances of the sulfonamide part of the dipeptide conjugates **1**–**30** were observed at 8.10–8.33 ppm region as triplet peak in the ^1H NMR spectrum. Other amide NH resonances of the dipeptide-sulfonamide conjugates were observed at 7.67–8.29 ppm region as doublet or triplet depending on the amino acid unit. The carbamate NH resonances of the dipeptide-sulfonamide conjugates were observed at 6.96–7.62 ppm region whereas NH_2 resonances of sulfonamide part were observed generally in the aromatic region together with aromatic protons. These NH or NH_2 protons were confirmed by D_2O exchange. The singlet that peaks around 5.00 ppm for compounds **1**–**21** was assigned to the CH_2



23-26

27)R= CH_2Ph, R'= $CH_2CH_2SCH_3$
28)R= CH_2Ph, R'= $CH(CH_3)CH_2CH_3$
29)R= CH_2Ph, R'= $CH_2CH(CH_3)_2$
30)R= CH_2Ph, R'= CH_2Ph

Scheme 2. Synthetic pathways of unprotected dipeptide-benzimidazole conjugates, 27–30.

protons for benzyloxycarbonyl protected group whereas the upfield singlet that signals around 1.30 ppm was assigned to the tert-butyl protons of Boc-protected group for compounds, **22**–**26**. Since Boc protected group was removed from compounds **27**–**30**, these peaks disappear in the spectra. Free NH_2 resonances of the unprotected dipeptide part were left under water peak in the DMSO solvent except for compound 28 which was observed at 1.74 ppm as a broad singlet. It is also mentioned that chemical shift values of the aliphatic peaks were shifted to high field, approximately 0.5 ppm. Carbonyl resonances of the amide carbonyls and carbamate carbonyl were observed around 172, 170 and 156 ppm, respectively. Carbonyl resonances of the unprotected amino acid-sulfonamide conjugates for compounds **27**–**30** were observed at 174 and 171 ppm. Due to unprotection, the peak around 156 ppm disappeared for compounds **27**–**28**. All other aliphatic and aromatic protons and carbons were observed in the expected regions (see Materials and Methods). The molecular ion or appropriate positive or negative ion peaks were observed for all proposed structures of novel compounds in the mass spectra. The IR spectra of dipeptide-sulfonamide conjugates, **1**–**26,** showed characteristic amide carbonyl peaks around between 1651 and 1710 cm^{-1}, whereas the carbamate carbonyl peaks around between 1633 and 1654 cm^{-1}. All other spectral data were in accordance with the assumed structures. As can be accepted, the carbamate carbonyl peak for compounds **27**–**30** was not observed due to unprotection. The amide carbonyl peaks for compounds **27**–**30** were observed at 10–15 cm^{-1} higher field than corresponding *N*-protected dipeptide-sulfonamide conjugates (between 1638 and 1682 cm^{-1}).

2.2. Carbonic anhydrase inhibition

Sulfonamide derivatives are an important pharmacophore in modern drug discovery and continue to be the most versatile class of compounds possessing different pharmacological activities such as antibacterial [24], anticancer [9,25,26], antiglaucoma [27], antiviral [9], anti-inflammatory [27], antioxidant [5] and carbonic anhydrase inhibitor [6–9]. Among the biological activities, human carbonic anhydrase (hCA, EC 4.2.1.1) inhibition has been the subject of several investigations since the discovery of the biological importance of this enzyme in several living organisms [28]. Considering the fact that primary sulfonamides and their isosteres are the main type of CA inhibitor compounds, we synthesized novel type dipeptide-sulfonamide conjugates to explore their possible carbonic anhydrase enzyme inhibition capacities against human carbonic anhydrase hCA I, II, IV and XII.

All the synthetized dipeptide conjugates have been evaluated by means of a stopped flow CO_2 hydrase assay [29] to test their inhibitory potency against four human (h) CA isoforms (hCA I, hCA II, hCA IV and hCA XII) [30–37]. Inhibition data are reported in Table 1, along with those referred to acetazolamide (AAZ), used as standard sulfonamide inhibitor. In order to evaluate the effect of *N*-protection on CA inhibitory potency, four unprotected dipeptide-sulfonamide conjugates (compounds **27**–**30**) have been prepared from the corresponding *N*-protected counterparts (**26**, **25**, **24** and **23**, respectively). The following structure–activity relationship (SAR) has been delineated:

(i) As for CA I, almost all the compounds showed Ki values in the medium nanomolar range, except for compounds **19**–**22**, that gave Ki values ranging from 4191.0 nM to 7015.0 nM. Among the synthesized dipeptide-sulfonamide conjugates, compounds **3**–**7**, **9**–**15**, **17**, **27** and **30** showed better inhibitory activity when compared to AAZ (Table 1). Compounds with alanine (**3**–**7**) or phenylalanine (**9**–**15**) as *N*-terminal residue gave the best inhibition values among the series, except for those with methionine at C-terminal position (compounds **8** and **16**). Indeed, the presence of this amino acid in the dipeptides led usually to a fall of inhibition activity. The contemporary presence of methionine and phenylalanine in the compounds **16**, **20**, **26** and **27** revealed to be also detrimental for the inhibitory potency as well as the shift from benzyloxycarbamate to Boc *N*-protecting group, leading to compounds **22**–**26** with higher Ki values when compared to **9, 11**, **13**, **14** and **16,** respectively. No crucial differences can be highlighted between *N*-protected and unprotected dipeptides, except for compound **30**, in which the removal of Boc residue led to a clear enhancement of inhibitory potency (73.2 vs 461.9).

Table 1
Inhibition data of hCA I, hCA II, hCA IV and hCA XII with compounds **1**–**30** and the standard sulfonamide inhibitor acetazolamide (**AAZ**) by a stopped flow CO_2 hydrase assay.

Cmp	K_I* (nM)			
	hCA I	hCA II	hCA IV	hCA XII
1	435.8	28.0	2467.8	7.21
2	831.6	34.2	626.9	51.1
3	91.5	6.3	702.1	48.5
4	82.3	70.7	398.1	52.6
5	70.4	374.6	2187.7	89.9
6	72.1	659.7	4504.6	761.1
7	64.5	14.3	52.2	17.8
8	824.8	41.8	38.3	49.8
9	64.6	41.5	34.3	6.1
10	81.0	53.8	225.7	5.6
11	86.0	42.1	35.4	8.9
12	73.2	25.8	21.7	53.1
13	78.7	7.4	15.4	8.9
14	61.0	9.1	15.0	8.0
15	74.2	6.1	14.4	7.1
16	398.6	2.8	7736.4	4.3
17	207.6	21.1	7893.8	362.2
18	256.3	6.0	518.5	7.7
19	7015.0	20.3	897.9	18.2
20	4281.0	51.2	> 10,000	67.6
21	4191.0	95.5	5423.5	68.9
22	5336.0	54.2	512.4	7.1
23	461.9	1.7	565.6	18.9
24	325.0	13.0	218.9	9.6
25	634.6	4.3	73.6	7.8
26	310.9	5.5	87.3	7.7
27	188.9	2.1	48.2	6.9
28	441.0	1.5	46.7	4.0
29	298.5	2.1	24.5	9.2
30	73.2	1.7	14.0	1.5
AAZ	250	12	74.2	5.7

* Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5–10% of the reported values).

(ii) All the synthetized dipeptides revealed to be highly potent and selective against CA II, with Ki values in the low nanomolar range for almost all the compounds. The unprotected dipeptides (compounds **27**–**30**) showed the best Ki values among the series, ranging from 1.5 nM and 2.1 nM, although the differences with the precursor compounds are not so dramatic (Table 1).

(iii) As shown in Table 1, the inhibition profile of the considered dipeptides against CA IV revealed to be quite similar to that shown towards CA I. Indeed, the data highlighted a detrimental effect on the inhibitory potency due to the presence of methionine in compounds **16**, **18**–**21** and a clear worsening of Ki values when benzyloxycarbamate is replaced with Boc as *N*-protecting group (compounds **22**–**25**). The only exception is represented by compound **26**, that showed a 88 fold enhancement of the inhibition potency, when compared to compound **16**. Compounds **12**–**15** showed the best Ki values among the series, along with the unprotected dipeptides, with Ki values ranging from 14.0 nM to 48.2 nM.

(iv) As for the tumor associated isoform CA XII, it revealed to be highly inhibited by all compounds here reported. Four derivatives **(10**, **16**, **28** and **30)** are even more potent than AAZ, with a Ki value of 1.5 nM shown by compound **30**, the most potent among the series.

As shown in Table 1, almost all the compounds showed Ki values in the low nanomolar range, with the only exception represented by compounds **6** (761.1 nM) and **17** (362.2 nM).

According to the obtained results, unprotected dipeptide-sulfonamide conjugates generally exhibited better activity than corresponding *N*-protected dipeptide-sulfonamide conjugates.

The observed good inhibitory properties against isoforms hCA II and XII, and the less efficient inhibition of isoforms hCA I and IV may be explained considering the tail approach, proposed by some of us some time ago [30,31]. Indeed, the dipeptide tails of the elongated molecules designed and reported here are able to interact with the entrance of the active site cavity, which is the most non-conserved part among the many CA isoforms known to date [32]. Presumably, in the case of isoforms II and XII, favorable interactions are prevalent, explaining the good observed affinities, whereas in the case of hCA I and IV some clashes may occur which might explain the less efficient inhibition, as reported for many CA – sulfonamide adducts characterized by means of X-ray crystallographic techniques [33].

3. Conclusions

A series of novel dipeptide-sulfonamide conjugates was synthesized by using *N*-protected dipeptide and 4-(aminoethyl)benzenesulfonamide. The carbonic anhydrase inhibitory properties of the novel compounds were determined using stop flow instruments. Most of the compounds exhibited potent inhibitory activity against hCA I, II, IV or XII. It is noteworthy that unprotected dipeptide-sulfonamide derivatives, **27**–**30** exhibit better inhibition activity than corresponding *N*-protected dipeptide-sulfonamide counterparts.

4. Material and methods

4.1. Chemistry

Anhydrous solvents and all reagents were purchased from Sigma-Aldrich (Milan, Italy), Acros (Milan, Italy) and Merck (Florence, Italy). Nuclear magnetic resonance (^{1}H NMR, ^{13C} NMR) spectra were recorded using a Bruker Advance III 400 MHz spectrometer in DMSO-d_6. Chemical shifts are reported in parts per million (ppm) and the coupling constants (J) are expressed in Hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; brs, broad singlet; dd, double of doubles. The assignment of exchangeable protons (OH and NH) was confirmed by the addition of D_2O. Positive or negative-ion electrospray ionization (ESI) mass spectra were recorded on a double-focusing Finnigan MAT 95 instrument with BE geometry. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F-254 plates. All microwave-assisted reactions were carried out in a microwave oven system manufactured by Milestone (Milestone Start S Microwave Labstation for Synthesis). Infrared spectra were recorded as KBr pellets in the range 4000–400 cm^{-1} on a Perkin Elmer FT-IR spectrophotometer. Elemental analyses were performed with a LECO CHNS-932 elemental analyzer. Melting points (mp) were measured in open capillary tubes and are uncorrected, using a Gallenkamp MPD350.BM3.5 apparatus. All starting *N*-protected dipeptides were prepared according to literature procedures [34].

4.2. General procedure for the synthesis of peptide-sulfonamide conjugates, 1–26

A mixture of equivalent amounts of the appropriate *N*-protected aminoacylbenzotriazole and 4-(2-aminoethyl)benzenesulfonamide was subjected to microwave irradiation (100 W, 70 °C) in anhydrous THF (5 mL) for 30 min. After completion of the reaction (using TLC plate), all volatiles were removed by rotavapor and the obtained crude product was crystallized from ethanol.

4.3. Benzyl (2-oxo-2-((2-oxo-2-((4-sulfamoylphenethyl)amino)ethyl)amino)ethyl)carbamate (1)

White solid (75%); mp 174–175 °C;^{1}H NMR (300 MHz, DMSO-d_6): δ 8.18 (t, 1H, CON*H*CH$_2$CH$_2$, J = 4.5 Hz), 7.96 (t, 1H, OCONHCH$_2$CON*H*, J = 4.5 Hz), 7.75 (d, 2H, Ar—*H*, J = 9.0 Hz), 7.56 (t, 1H, OCON*H*CH$_2$, J = 6.0 Hz), 7.41–7.31 (m, 9H, Ar—*H* + N*H*$_2$), 5.04 (s, 2H, C*H*$_2$OCO), 3.67 (d, 4H, C*H*$_2$CONHC*H*$_2$CO, J = 6.0 Hz), 3.34–3.28 (m, 2H, NHC*H*$_2$CH$_2$), 2.79 (t, 2H, NHCH$_2$C*H*$_2$, J = 7.5 Hz). ^{13}C NMR (300 MHz, DMSO-d_6): δ 169.4 (OCONHCH$_2$*C*O), 168.7 (*C*ONHCH$_2$CH$_2$), 156.5 (CH$_2$O*C*O), 143.5, 142.0, 137.0, 129.1, 128.3, 127.8, 127.7, 125.7 (Ar—*C*), 65.5 (*C*H$_2$OCO), 43.6 (CH$_2$OCONH*C*H$_2$CO), 42.0 (*C*H$_2$CONHCH$_2$CH$_2$), 34.7 (CONHCH$_2$*C*H$_2$). $_{\nu}$(C=O)carbamate: 1644 cm^{-1}, $_{\nu}$(C=O)amide: 1656, 1684 cm^{-1}, $_{\nu}$(N—H)amine: 3270, 3336 cm^{-1}. Anal. calculated for $C_{20}H_{24}N_4O_6S$: C, 53.56; H, 5.39; N, 12.49; S, 7.15. Found: C, 52.63; H, 5.09; N, 12.07; S, 7.01. HRMS *m/z* for $C_{20}H_{24}N_4O_6S$ $[M + H]^+$ calcd. 449.1, found 449.2; $[M + Na]^+$ calcd. 471.1, found 422.2; $[M + HCOOH]^+$ calcd. 494.1, found 494.2; $[M-H]^-$ calcd. 447.1, found 447.0; $[M + Cl]^-$, calcd. 483.1, found 483.2; $[M + HCOO]^-$, calcd. 493.1, found 493.1.

4.4. Benzyl (R)-(2-((4-(methylthio)-1-oxo-1-((4-sulfamoylphenethyl)amino)butan-2-yl)amino)-2-oxoethyl)carbamate (2)

White solid (94%); mp 188–189 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.07–8.05 (m, 2H, N*H*CHCON*H*CH$_2$CH$_2$), 7.74 (d, 2H, Ar—*H*, J = 8.0 Hz), 7.49 (t, 1H, OCON*H*CH$_2$, J = 3.0 Hz), 7.39–7.29 (m, 9H, Ar—*H* + N*H*$_2$), 5.04 (s, 2H, CH$_2$OCO),4.33–4.27 (m, 1H, C*H*CH$_2$CH$_2$SCH$_3$), 3.67 (d, 2H, OCONHC*H*$_2$CO, J = 4.0 Hz), 3.35–3.25 (m, 2H, NHC*H*$_2$CH$_2$), 2.79 (t, 2H, NHCH$_2$C*H*$_2$, J = 6.0 Hz), 2.45–2.32 (m, 2H, CHCH$_2$C*H*$_2$SCH$_3$), 2.01 (s, 3H, CHCH$_2$CH$_2$SC*H*$_3$), 1.90–1.70 (m, 2H, CHC*H*$_2$CH$_2$SCH$_3$). ^{13}C NMR (400 MHz, DMSO-d_6): δ 170.4 (OCONHCH$_2$*C*O), 168.5 (*C*ONHCH$_2$CH$_2$), 156.9 (CH$_2$O*C*O), 143.0, 141.5, 136.4, 128.5, 127.7, 127.1, 125.1 (Ar—*C*), 64.9 (*C*H$_2$OCO), 51.2 (*C*HCH$_2$CH$_2$SCH$_3$), 42.9 (OCONH*C*H$_2$CO), 34.1 (CONHCH$_2$*C*H$_2$), 31.2 (CHCH$_2$*C*H$_2$SCH$_3$), 28.9 (CHCH$_2$CH$_2$S*C*H$_3$), 14.0 (CH*C*H$_2$CH$_2$SCH$_3$). $_{\nu}$(C=O) carbamate: 1644 cm^{-1}, $_{\nu}$(C=O)amide: 1690, 1710 cm^{-1}, $_{\nu}$(N—H)amine: 2968, 3288 cm^{-1}. Anal. calculated for $C_{23}H_{30}N_4O_6S_2$: C, 52.86; H, 5.79; N, 10.72; S, 12.27. Found: C, 52.20; H, 5.19; N, 10.34; S, 11.47. HRMS *m/z* for $C_{23}H_{30}N_4O_6S_2$ $[M + H]^+$ calcd. 523.2, found 523.2; $[M + Na]^+$ calcd. 545.2, found 545.1; $[M + HCOOH]^+$ calcd. 568.2, found 568.2; $[M-H]^-$ calcd. 521.2, found 521.1; $[M + Cl]^-$, calcd. 557.1, found 557.1.

4.5. Benzyl (S)-(1-oxo-1-((2-oxo-2-((4-sulfamoylphenethyl)amino)ethyl)amino)propan-2-yl)carbamate (3)

White solid (73%); mp 152–153 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.19 (t, 1H, N*H*CH$_2$CH$_2$, J = 4.5 Hz), 7.84 (t, 1H, OCONHCHCON*H*, J = 6.0 Hz), 7.74 (d, 2H, Ar—*H*, J = 6.0 Hz), 7.60 (d, 1H, OCON*H*CH, J = 6.0 Hz), 7.43–7.28 (m, 9H, Ar—*H* + N*H*$_2$), 5.08–4.99 (m, 2H, C*H*$_2$OCO), 4.07–4.03 (m, 1H, OCONHC*H*), 3.65 (d, 2H, NHC*H*$_2$CONHCH$_2$CH$_2$, J = 6.0 Hz) 3.34–3.27 (m, 2H, NHC*H*$_2$CH$_2$), 2.79 (t, 2H, NHCH$_2$C*H*$_2$, J = 7.5 Hz), 1.22 (d, 3H, C*H*$_3$, J = 9.0 Hz). ^{13}C NMR (300 MHz, DMSO-d_6): δ 172.8 (OCONHCH*C*O), 168.6 (*C*ONHCH$_2$CH$_2$), 156.0 (CH$_2$O*C*O), 143.5, 142.0, 136.9, 129.1, 128.3, 127.8, 127.7, 125.7 (Ar—*C*), 65.4 (*C*H$_2$OCO), 50.2 (*C*HCH$_3$), 42.1 (*C*H$_2$CONHCH$_2$CH$_2$), 34.7 (CONHCH$_2$*C*H$_2$), 17.8 (*C*H$_3$). $_{\nu}$(C=O)carbamate: 1641 cm^{-1}, $_{\nu}$(C=O)amide: 1686 cm^{-1}, $_{\nu}$(N—H)amine: 3297 cm^{-1}. Anal. calculated for $C_{21}H_{26}N_4O_6S$: C, 54.53; H, 5.67; N, 12.11; S, 6.93. Found: C, 54.14; H, 5.35; N, 12.39; S, 7.14. HRMS *m/z* for $C_{21}H_{26}N_4O_6S$ $[M + H]^+$ calcd. 463.2, found 463.2; $[M + Na]^+$ calcd. 485.1, found 485.1; $[M + HCOOH]^+$ calcd. 508.2, found 508.2; $[M-H]^-$ calcd. 461.1, found 461.1; $[M + Cl]^-$, calcd. 497.1, found 497.2.

4.6. Benzyl ((S)-1-oxo-1-(((S)-1-oxo-1-((4-sulfamoylphenethyl)amino)propan-2-yl)amino)propan-2-yl)carbamate (*4*)

White solid (83%); mp 221–222 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.99–7.94 (m, 2H, N*H*CHCON*H*CH$_2$CH$_2$), 7.74 (d, 2H, Ar–*H*, *J* = 9.0 Hz), 7.50 (d, 1H, OCON*H*CH, *J* = 6.0 Hz), 7.40–7.31 (m, 9H, Ar–*H* + NH_2), 5.03 (s, 2H, CH_2OCO), 4.24–4.15 (m, 1H, OCONHCHCONHC*H*), 4.08–4.03 (m, 2H, OCONHC*H*CONHCH), 3.36–3.23 (m, 2H, NHCH_2CH$_2$), 2.78 (t, 2H, NHCH$_2$CH_2, *J* = 7.5 Hz), 1.20–1.14 (m, 6H, CH_3 + CH_3). ^{13}C NMR (300 MHz, DMSO-d_6): δ 172.0 (OCONHCH*C*ONHCH*C*O), 155.7 (CH$_2$O*C*O), 143.5, 142.1, 137.0, 129.1, 128.3, 127.8, 127.7, 125.6 (Ar–*C*), 65.3 (*C*H$_2$OCO), 50.0 (OCONHCHCONH*C*H), 48.1 (OCONH*C*HCONHCH), 34.6 (CONHCH$_2$*C*H$_2$), 18.4 (*C*H$_3$), 18.0 (*C*H$_3$). ν(C=O)carbamate: 1633 cm^{-1}, ν(C=O)amide: 1656, 1693 cm^{-1}, ν(N–H)amine: 3312 cm^{-1}. Anal. calculated for $\mathrm{C_{22}H_{28}N_4O_6S}$: C, 55.45; H, 5.92; N, 11.76; S, 6.73. Found: C, 55.63; H, 5.53; N, 11.24; S, 6.52. HRMS *m/z* for $\mathrm{C_{22}H_{28}N_4O_6S}$ $[\mathrm{M + H}]^+$ calcd. 477.2, found 477.2; $[\mathrm{M + Na}]^+$ calcd. 499.2, found 499.2; $[\mathrm{M + HCOOH}]^+$ calcd. 522.2, found 522.2; $[\mathrm{M - H}]^-$ calcd. 475.2, found 474.5; $[\mathrm{M + Cl}]^-$, calcd. 511.1, found 511.2; $[\mathrm{M + HCOO}]^-$, calcd. 522.2, found 521.1.

4.7. Benzyl ((S)-1-oxo-1-(((S)-1-oxo-3-phenyl-1-((4-sulfamoylphenethyl)amino)propan-2-yl)amino)propan-2-yl)carbamate (*5*)

White solid (72%); mp 219–220 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.10 (t, 1H, N*H*CH$_2$CH$_2$, *J* = 6.0 Hz), 7.99 (d, 1H, OCONHCHCON*H*, *J* = 9.0 Hz), 7.73 (d, 2H, Ar–*H*, *J* = 6.0 Hz), 7.47 (d, 1H, OCON*H*CH, *J* = 6.0 Hz), 7.36–7.17 (m, 14H, Ar–*H* + NH$_2$), 5.02 (s, 2H, CH_2OCO), 4.47–4.39 (m, 1H, OCONHCHCONHC*H*), 4.06–3.97 (m, 1H, OCONH-C*H*CONHCH), 3.36–3.21 (m, 2H, NHCH_2CH$_2$), 2.97–2.71 (m, 4H, PhCH_2CH + NHCH$_2$CH_2), 1.13 (d, 3H, CH_3, *J* = 9.0 Hz). ^{13}C NMR (300 MHz, DMSO-d_6): δ 172.1 (OCONHCH*C*O), 170.7 (*C*ONHCH$_2$CH$_2$), 155.7 (CH$_2$O*C*O), 143.5, 142.1, 137.6, 136.9, 129.2, 129.1, 128.3, 128.0, 127.8, 127.7, 126.2, 125.6 (Ar–*C*), 65.4 (*C*H$_2$OCO), 50.8 (OCONHCHCONH*C*H), 50.2 (OCONH*C*HCONHCH), 37.7 (Ph*C*H$_2$CH), 34.6 (CONHCH$_2$*C*H$_2$), 18.0 (*C*H$_3$). ν(C=O)carbamate: 1633 cm^{-1}, ν(C=O)amide: 1660, 1693 cm^{-1}, ν(N–H)amine: 3316 cm^{-1}. Anal. calculated for $\mathrm{C_{28}H_{32}N_4O_6S}$: C, 60.85; H, 5.84; N, 10.14; S, 5.80. Found: C, 60.43; H, 4.90; N, 9.32; S, 5.13. HRMS *m/z* for $\mathrm{C_{28}H_{32}N_4O_6S}$ $[\mathrm{M + H}]^+$ calcd. 553.2, found 553.3; $[\mathrm{M + Na}]^+$ calcd. 575.2, found 575.2; $[\mathrm{M + HCOOH}]^+$ calcd. 598.2, found 598.2; $[\mathrm{M + Cl}]^-$, calcd. 587.2, found 587.2.

4.8. Benzyl ((S)-1-(((R)-3-(benzylthio)-1-oxo-1-((4-sulfamoylphenethyl)amino)propan-2-yl)amino)-1-oxopropan-2-yl)carbamate (*6*)

White solid (86%); mp 194–195 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.21 (t, 1H, N*H*CH$_2$CH$_2$, *J* = 4.0 Hz), 8.08 (d, 1H, OCONHCHCON*H*, *J* = 8.0 Hz), 7.73 (d, 2H, Ar–*H*, *J* = 8.0 Hz), 7.53 (d, 1H, OCON*H*CH, *J* = 4.0 Hz), 7.40–7.21 (m, 14H, Ar–*H* + NH_2), 5.03 (s, 2H, CH_2OCO), 4.48–4.42 (m, 1H, OCONHCHCONHC*H*), 4.13–4.06 (m, 1H, OCONH-C*H*CONHCH), 3.74 (s, 2H, CHCH$_2$SCH_2Ph) 3.35–3.30 (m, 2H, NHCH_2CH$_2$), 2.79 (t, 2H, NHCH$_2$CH_2, *J* = 6.0 Hz), 2.71–2.54 (m, 2H, CHCH_2SCH$_2$Ph), 1.21 (d, 3H, CH_3, *J* = 8.0 Hz).

^{13}C NMR (400 MHz, DMSO-d_6): δ 172.9 (OCONHCH*C*O), 170.4 (*C*ONHCH$_2$CH$_2$), 156.3 (CH$_2$O*C*O), 144.0, 142.6, 138.8, 137.4, 129.6, 129.4, 128.8, 128.3, 128.2, 127.3, 126.1 (Ar–*C*), 65.9 (*C*H$_2$OCO), 52.7 (OCONHCHCONH*C*H), 50.7 (OCONH*C*HCONHCH), 35.6 (CHCH$_2$S*C*H$_2$Ph), 35.1 (CONHCH$_2$*C*H$_2$), 33.4 (CH*C*H$_2$SCH$_2$Ph), 18.6 (*C*H$_3$). ν(C=O)carbamate: 1639 cm^{-1}, ν(C=O)amide: 1675 cm^{-1}, ν(N–H)amine: 3295 cm^{-1}. Anal. calculated for $\mathrm{C_{29}H_{34}N_4O_6S_2}$: C, 58.18; H, 5.72; N, 9.36; S, 10.71. Found: C, 57.81; H, 5.548; N, 9.257; S, 10.50. HRMS *m/z* for $\mathrm{C_{29}H_{34}N_4O_6S_2}$ $[\mathrm{M + H}]^+$ calcd. 599.2, found 599.3; $[\mathrm{M + Na}]^+$ calcd. 621.2, found 621.2; $[\mathrm{M + HCOOH}]^+$ calcd. 644.2, found 644.2; $[\mathrm{M + Cl}]^-$, calcd. 633.2, found 633.2.

4.9. Benzyl ((S)-1-(((S)-3-methyl-1-oxo-1-((4-sulfamoylphenethyl)amino)butan-2-yl)amino)-1-oxopropan-2-yl)carbamate (*7*)

White solid (84%); mp 231–232 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.13 (t, 1H, N*H*CH$_2$CH$_2$, *J* = 4.5 Hz), 7.73 (d, 2H, Ar–*H*, *J* = 9.0 Hz), 7.67 (d, 1H, OCONHCHCON*H*, *J* = 9.0 Hz), 7.53 (d, 1H, OCON*H*CH, *J* = 9.0 Hz), 7.40–7.30 (m, 9H, Ar–*H* + NH_2), 5.02 (s, 2H, CH_2OCO), 4.14–4.06 (m, 2H, OCONHC*H*CONHC*H*), 3.41–3.27 (m, 2H, NHCH_2CH$_2$), 2.79 (t, 2H, NHCH$_2$CH_2, *J* = 6.0 Hz), 1.89–1.83 (m, 1H, C*H*(CH$_3$)$_2$), 1.19 (d, 3H, CH_3, *J* = 6.0 Hz), 0.77 (d, 6H, CH(CH_3)$_2$, *J* = 6.0 Hz). ^{13}C NMR (300 MHz, DMSO-d_6): δ 172.3 (OCONHCH*C*O), 170.7 (*C*ONHCH$_2$CH$_2$), 155.6 (CH$_2$O*C*O), 143.5, 142.0, 137.0, 129.1, 128.3, 127.7, 127.7, 125.6 (Ar–*C*), 65.3 (*C*H$_2$OCO), 57.5 (OCONHCHCONH*C*H), 50.1 (OCONH*C*HCONHCH), 39.6 (NH*C*H$_2$CH$_2$) 34.6 (CONHCH$_2$*C*H$_2$), 30.8 (*C*H(CH$_3$)$_2$), 19.1 (*C*H$_3$), 18.1 and 18.0 (CH(*C*H$_3$)$_2$). ν(C=O)carbamate: 1637 cm^{-1}, ν(C=O)amide: 1660, 1694 cm^{-1}, ν(N–H)amine: 3323 cm^{-1}. Anal. calculated for $\mathrm{C_{24}H_{32}N_4O_6S}$: C, 57.13; H, 6.39; N, 11.10; S, 6.35. Found: C, 57.20; H, 5.94; N, 10.55; S, 6.10. HRMS *m/z* for $\mathrm{C_{24}H_{32}N_4O_6S}$ $[\mathrm{M + H}]^+$ calcd. 505.2, found 505.3; $[\mathrm{M + Na}]^+$ calcd. 527.2, found 527.2; $[\mathrm{M + HCOOH}]^+$ calcd. 550.2, found 550.2; $[\mathrm{M - H}]^-$, calcd. 503.2, found 503.1; $[\mathrm{M + Cl}]^-$, calcd. 539.2, found 533.3.

4.10. Benzyl ((S)-1-(((S)-4-(methylthio)-1-oxo-1-((4-sulfamoylphenethyl)amino)butan-2-yl)amino)-1-oxopropan-2-yl)carbamate (*8*)

White solid (88%); mp 184–185 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.01–7.97 (m, 2H, N*H*CH$_2$CH$_2$ + OCONHCHCON*H*), 7.73 (d, 2H, Ar–H, *J* = 8.0 Hz), 7.51 (bs, 1H, OCON*H*CH), 7.36–7.16 (m, 9H, Ar–*H* + NH_2), 5.03 (s, 2H, CH_2OCO), 4.29–4.28 (m, 1H, OCONHCHCONHC*H*), 4.07 (bs, 1H, OCONHC*H*CONHCH), 3.39–3.25 (m, 2H, NHCH_2CH$_2$), 2.78 (t, 2H, NHCH$_2$CH_2, *J* = 6.0 Hz), 2.46–2.32 (m, 2H, CHCH$_2$CH_2SCH$_3$), 2.01 (s, 3H, CHCH$_2$CH$_2$SCH_3), 1.96–1.73 (m, 2H, CHCH_2CH$_2$SCH$_3$), 1.20 (d, 3H, CH_3, *J* = 8.0 Hz).

^{13}C NMR (400 MHz, DMSO-d_6): δ 171.9 (OCONHCH*C*O), 170.3 (*C*ONHCH$_2$CH$_2$), 155.2 (CH$_2$O*C*O), 142.8, 141.8, 136.4, 128.5, 127.7, 127.2, 127.1, 125.0 (Ar–*C*), 64.8 (*C*H$_2$OCO), 51.2 (OCONHCHCONH*C*H), 49.6 (OCONH*C*HCONHCH), 39.2 (NH*C*H$_2$CH$_2$) 34.0 (CONHCH$_2$*C*H$_2$), 31.4 (CH*C*H$_2$CH$_2$SCH$_3$), 28.9 (CHCH$_2$*C*H$_2$SCH$_3$), 17.3 (*C*H$_3$), 14.0 (CHCH$_2$CH$_2$S*C*H$_3$). ν(C=O)carbamate: 1640 cm^{-1}, ν(C=O)amide: 1686 cm^{-1}, ν(N–H)amine: 3280, 3675 cm^{-1}. Anal. calculated for $\mathrm{C_{24}H_{32}N_4O_6S_2}$: C, 53.71; H, 6.01; N, 10.44; S, 11.95. Found: C, 52.80; H, 5.685; N, 9.765; S, 10.96. HRMS *m/z* for $\mathrm{C_{24}H_{32}N_4O_6S_2}$ $[\mathrm{M + H}]^+$ calcd. 505.2, found 505.3; $[\mathrm{M + Na}]^+$ calcd. 527.2, found 527.2; $[\mathrm{M + HCOOH}]^+$ calcd. 550.2, found 550.2; $[\mathrm{M + Cl}]^-$, calcd. 539.2, found 533.3.

4.11. Benzyl (S)-(1-oxo-1-((2-oxo-2-((4-sulfamoylphenethyl)amino)ethyl)amino)-3-phenylpropan-2-yl)carbamate (*9*)

White solid (87%); mp 190–191 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.33 (t, 1H, N*H*CH$_2$CH$_2$, *J* = 6.0 Hz), 7.86 (t, 1H, OCONHCHCON*H*, *J* = 6.0 Hz), 7.76 (d, 2H, Ar–*H*, *J* = 9.0 Hz), 7.61 (d, 1H, OCON*H*CH, *J* = 9.0 Hz), 7.41 (d, 2H, Ar–*H*, *J* = 9.0 Hz), 7.37–7.19 (m, 12H, Ar–*H* + NH_2), 4.96 (s, 2H, CH_2OCO), 4.32–4.25 (m, 1H, OCONHC*H*-CO), 3.69 (t, 2H, OCONHCHCONHCH_2, *J* = 6.0 Hz), 3.35–3.29 (m, 2H, NHCH_2CH$_2$), 3.08–3.02 (m, 1H, PhCH_2CH), 2.82–2.77 (m, 3H, PhCH_2CH + NHCH$_2$CH_2). ^{13}C NMR (300 MHz, DMSO-d_6): δ 171.8 (OCONHCH*C*O), 168.6 (*C*ONHCH$_2$CH$_2$), 156.0 (CH$_2$O*C*O), 143.5, 142.0, 138.1, 136.9, 129.2, 129.1, 128.3, 128.0, 127.7, 127.4, 126.2, 125.7 (Ar–*C*), 65.2 (*C*H$_2$OCO), 56.2 (OCONH*C*HCONHCH$_2$), 42.1 (OCONHCHCONH*C*H$_2$), 37.2 (CH*C*H$_2$Ph), 34.7 (CONHCH$_2$*C*H$_2$). ν(C=O)carbamate: 1652 cm^{-1}, ν(C=O)amide: 1691 cm^{-1}, ν(N–H)amine: 3312, 3406, 3676 cm^{-1}. Anal. calculated for $\mathrm{C_{27}H_{30}N_4O_6S}$: C, 60.21; H, 5.61; N, 10.40; S, 5.95. Found: C, 59.95; H, 5.437; N, 10.36; S, 6.142. HRMS *m/z* for $\mathrm{C_{27}H_{30}N_4O_6S}$ $[\mathrm{M + H}]^+$ calcd. 539.2, found 539.2;

[M + Na]$^+$ calcd. 561.2, found 561.2; [M + HCOOH]$^+$ calcd. 584.2, found 584.2; [M + Cl]$^-$, calcd. 573.2, found 573.1.

4.12. Benzyl ((S)-1-oxo-1-(((S)-1-oxo-1-((4-sulfamoylphenethyl)amino)propan-2-yl)amino)-3-phenylpropan-2-yl)carbamate (*10*)

White solid (92%); mp 190–191 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.15 (d, 1H, OCONHCHCON*H*, J = 6.0 Hz), 7.96 (t, 1H, N*H*CH$_2$CH$_2$, J = 6.0 Hz), 7.75 (d, 2H, Ar—*H*, J = 9.0 Hz), 7.53 (d, 1H, OCON*H*CH, J = 9.0 Hz), 7.41–7.18 (m, 14H, Ar—*H* + N*H$_2$*), 4.94 (s, 2H, C*H$_2$*OCO), 4.32–4.20 (m, 2H, OCONHC*H*CONHC*H*), 3.37–3.24 (m, 2H, NHC*H$_2$*CH$_2$), 3.07–3.01 (m, 1H, PhC*H$_2$*CH), 2.81–2.68 (m, 3H, PhC*H$_2$*CH + NHCH$_2$C*H$_2$*), 1.18 (d, 3H, C*H$_3$*, J = 6.0 Hz). ^{13}C NMR (300 MHz, DMSO-d_6): δ 172.0 (OCONHCHC*O*), 171.1 (*C*ONHCH$_2$CH$_2$), 155.8 (CH$_2$O*C*O),143.6, 142.0, 138.1, 137.0, 129.2, 128.3, 128.0, 127.6, 127.4, 126.2, 125.6 (Ar—*C*), 65.1 (*C*H$_2$OCO), 56.0 (OCONHC-HCONHC*H*), 48.2 (OCONH*C*HCONHCH), 37.3 (CH*C*H$_2$Ph), 34.7 (CONHCH$_2$*C*H$_2$), 18.4 (*C*H$_3$). ν(C=O)carbamate: 1652 cm^{-1}, ν(C=O) amide: 1668, 1691 cm^{-1}, ν(N—H)amine: 3329, 3676 cm^{-1}. Anal. calculated for C$_{28}$H$_{32}$N$_4$O$_6$S: C, 60.85; H, 5.84; N, 10.14; S, 5.80. Found: C, 59.88; H, 6.019; N, 9.867; S, 5.570. HRMS *m/z* for C$_{28}$H$_{32}$N$_4$O$_6$S [M + H]$^+$ calcd. 553.2, found 553.3; [M + Na]$^+$ calcd. 575.2, found 575.2; [M + HCOOH]$^+$ calcd. 598.2, found 598.2; [M + Cl]$^-$, calcd. 587.2, found 587.2.

4.13. Benzyl ((S)-1-oxo-1-(((S)-1-oxo-3-phenyl-1-((4-sulfamoylphenethyl)amino)propan-2-yl)amino)-3-phenylpropan-2-yl)carbamate (*11*)

White solid (90%); mp 207–208 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.19 (d, 1H, OCONHCHCON*H*, J = 9.0 Hz), 8.12 (d, 1H, N*H*CH$_2$CH$_2$, J = 6.0 Hz), 7.75 (d, 2H, Ar—*H*, J = 6.0 Hz), 7.49 (d, 1H, OCON*H*CH, J = 9.0 Hz), 7.37–7.18 (m, 19H, Ar—*H* + N*H$_2$*), 4.96 (s, 2H, C*H$_2$*OCO), 4.53–4.45 (m, 1H, OCONHCHCONHC*H*), 4.27 (bs, 1H, OCONHC*H*CO-NHCH), 3.37–3.23 (m, 2H, NHC*H$_2$*CH$_2$), 2.97–2.71 (m, 6H, PhC*H$_2$*CH + PhC*H$_2$*CH + NHCH$_2$C*H$_2$*).

^{13}C NMR (300 MHz, DMSO-d_6): δ 171.1 (OCONHCHC*O*), 170.7 (*C*ONHCH$_2$CH$_2$), 155.7 (CH$_2$O*C*O),143.6, 141.9, 137.8, 137.4, 136.9, 129.2, 129.1, 128.3, 128.1, 128.0, 127.7, 127.3, 126.3, 126.2, 125.7 (Ar—*C*), 65.2 (*C*H$_2$OCO), 56.0 (OCONHCHCONHCH), 53.9 (OCONH*C*-HCONHCH), 39.7 (NH*C*H$_2$CH$_2$), 37.8 (CH*C*H$_2$Ph), 37.3 (CH*C*H$_2$Ph), 34.6 (CONHCH$_2$*C*H$_2$). ν(C=O)carbamate: 1638 cm^{-1}, ν(C=O)amide: 1697 cm^{-1}, ν(N—H)amine: 3302, 3676 cm^{-1}. Anal. calculated for C$_{34}$H$_{36}$N$_4$O$_6$S: C, 64.95; H, 5.77; N, 8.91; S, 5.10. Found: C, 64.50; H, 5.500; N, 8.768; S, 4.863. HRMS *m/z* for C$_{34}$H$_{36}$N$_4$O$_6$S [M + H]$^+$ calcd. 629.2, found 629.2; [M + Na]$^+$ calcd. 651.2, found 651.2; [M + Cl]$^-$, calcd. 663.2, found 663.2.

4.14. Benzyl ((S)-1-(((R)-3-(benzylthio)-1-oxo-1-((4-sulfamoylphenethyl)amino)propan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (*12*)

White solid (94%); mp 198–199 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.29–8.25 (m, 2H, OCONHCHCON*H* + N*H*CH$_2$CH$_2$), 7.74 (d, 2H, Ar—*H*, J = 8.0 Hz), 7.54 (d, 1H, OCON*H*CH, J = 12.0 Hz), 7.42–7.18 (m, 19H, Ar—*H* + N*H$_2$*), 4.95 (s, 2H, C*H$_2$*OCO), 4.52–4.47 (m, 1H, OCONHCHCONHC*H*), 4.37–4.31 (m, 1H, OCONHC*H*CONHCH), 3.76 (s, 2H, CH$_2$SC*H$_2$*Ph), 3.39–3.29 (m, 2H, NHC*H$_2$*CH$_2$), 3.04–3.00 (m, 1H, PhC*H$_2$*CH), 2.82–2.54 (m, 5H, PhC*H$_2$*CH + C*H$_2$*SCH$_2$Ph + NHCH$_2$C*H$_2$*). ^{13}C NMR (400 MHz, DMSO-d_6): δ 171.9 (OCONHCHC*O*), 170.4 (*C*ONHCH$_2$CH$_2$), 156.3 (CH$_2$O*C*O),144.1, 142.6, 138.8, 138.5, 129.7, 129.7, 129.5, 128.8, 128.8, 128.5, 128.1, 127.8, 127.3, 126.7, 126.2 (Ar—*C*), 65.7 (*C*H$_2$OCO), 56.6 (OCONHCHCONH*C*H), 52.8 (OCONH*C*-HCONHCH), 40.5 (NH*C*H$_2$CH$_2$), 38.0 (CH*C*H$_2$Ph), 35.6 (CH$_2$S*C*H$_2$Ph), 35.2 (CONHCH$_2$*C*H$_2$), 33.4 (*C*H$_2$SCH$_2$Ph). ν(C=O)carbamate: 1636 cm^{-1}, ν(C=O)amide: 1698 cm^{-1}, ν(N—H)amine: 3286 cm^{-1}. Anal. calculated for C$_{35}$H$_{38}$N$_4$O$_6$S$_2$: C, 62.29; H, 5.68; N, 8.30; S, 9.50. Found: C, 62.09; H, 5.629; N, 8.172; S, 9.302. HRMS *m/z* for C$_{35}$H$_{38}$N$_4$O$_6$S$_2$ [M + H]$^+$ calcd. 675.2, found 675.3; [M + Na]$^+$ calcd. 697.2, found 697.3; [M + Cl]$^-$, calcd. 709.2, found 709.3.

4.15. Benzyl ((S)-1-(((S)-4-methyl-1-oxo-1-((4-sulfamoylphenethyl)amino)pentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (*13*)

White solid (89%); mp 197–198 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.07–8.02 (m, 2H, OCONHCHCON*H* + N*H*CH$_2$CH$_2$), 7.74 (d, 2H, Ar—*H*, J = 6.0 Hz), 7.50 (d, 1H, OCON*H*CH, J = 6.0 Hz), 7.40–7.20 (m, 14H, Ar—*H* + N*H$_2$*), 4.95 (s, 2H, C*H$_2$*OCO), 4.29–4.25 (m, 2H, OCON-HC*H*CONHC*H*), 3.35–3.26 (m, 2H, NHC*H$_2$*CH$_2$), 3.04–2.99 (m, 1H, CHC*H$_2$*Ph), 2.80–2.76 (m, 3H, CHC*H$_2$*Ph + NHCH$_2$C*H$_2$*), 1.51–1.40 (m, 3H, CHC*H$_2$*C*H*(CH$_3$)$_2$), 0.88–0.81 (m, 6H, CH(C*H$_3$*)$_2$). ^{13}C NMR (300 MHz, DMSO-d_6): δ 171.8 (OCONHCHC*O*), 171.2 (*C*ONHCH$_2$CH$_2$), 155.8 (CH$_2$O*C*O),143.6, 142.0, 138.0, 137.0, 129.2, 129.1, 128.2, 128.0, 127.6, 127.4, 126.2, 125.6 (Ar—*C*), 65.1 (*C*H$_2$OCO), 56.0 (OCONH*C*HCONHCH), 51.1 (OCONHCHCONH*C*H), 41.2 (CH*C*H$_2$CH(CH$_3$)$_2$), 37.3 (CH*C*H$_2$Ph), 34.6 (CONHCH$_2$*C*H$_2$), 24.1 (CHCH$_2$*C*H(CH$_3$)$_2$), 22.9 and 21.7 (CHCH$_2$CH(*C*H$_3$)$_2$). ν(C=O)carbamate: 1639 cm^{-1}, ν(C=O)amide: 1696 cm^{-1}, ν(N—H)amine: 3305, 3676 cm^{-1}. Anal. calculated for C$_{31}$H$_{38}$N$_4$O$_6$S: C, 62.61; H, 6.44; N, 9.42; S, 5.39. Found: C, 61.92; H, 6.156; N, 9.517; S, 4.792. HRMS *m/z* for C$_{31}$H$_{38}$N$_4$O$_6$S [M + H]$^+$ calcd. 595.3, found 595.3; [M + Na]$^+$ calcd. 617.2, found 617.3; [M + Cl]$^-$, calcd. 629.2, found 629.3.

4.16. Benzyl ((S)-1-(((2S,3S)-3-methyl-1-oxo-1-((4-sulfamoylphenethyl)amino)pentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (*14*)

White solid (95%); mp 223–224 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.11 (t, 1H, N*H*CH$_2$CH$_2$, J = 4.5 Hz), 7.89 (d, 1H, OCONHCHCON*H*, J = 12.0 Hz), 7.75 (d, 2H, Ar—*H*, J = 9.0 Hz), 7.55 (d, 1H, OCON*H*CH, J = 9.0 Hz), 7.41–7.20 (m, 14H, Ar—*H* + N*H$_2$*), 4.96 (s, 2H, C*H$_2$*OCO), 4.36–4.31 (m, 1H, OCONHCHCONHC*H*), 4.15 (m, 1H, OCONHC*H*CO-NHCH), 3.42–3.26 (m, 2H, NHC*H$_2$*CH$_2$), 3.03–2.98 (m, 1H, CHC*H$_2$*Ph), 2.82–2.71 (m, 3H, CHC*H$_2$*Ph + NHCH$_2$C*H$_2$*), 1.66 (bs, 1H, CH(CH$_3$)C*H$_2$*CH$_3$), 1.38 (bs, 1H, CH(CH$_3$)C*H$_2$*CH$_3$), 1.09–0.99 (m, 1H, C*H*(CH$_3$)CH$_2$CH$_3$), 0.82–0.75 (m, 6H, CH(C*H$_3$*)CH$_2$C*H$_3$*). ^{13}C NMR (300 MHz, DMSO-d_6): δ 171.2 (OCONHCHC*O*), 170.7 (*C*ONHCH$_2$CH$_2$), 155.8 (CH$_2$O*C*O),143.6, 142.0, 138.0, 137.0, 129.2, 129.1, 128.2, 128.0, 127.6, 127.3, 126.2, 125.6 (Ar—*C*), 65.1 (*C*H$_2$OCO), 56.8 (OCONHCHCONH*C*H), 56.0 (OCONH*C*HCONHCH), 37.3 (CH*C*H$_2$Ph), 36.8 (*C*H(CH$_3$)CH$_2$CH$_3$), 34.6 (CONHCH$_2$*C*H$_2$), 24.2 (CH(CH$_3$)*C*H$_2$CH$_3$), 15.2 (CH(*C*H$_3$)CH$_2$CH$_3$), 11.0 (CH(CH$_3$)CH$_2$*C*H$_3$). ν(C=O)carbamate: 1630 cm^{-1}, ν(C=O)amide: 1698 cm^{-1}, ν(N—H)amine: 3290, 3676 cm^{-1}. Anal. calculated for C$_{31}$H$_{38}$N$_4$O$_6$S: C, 62.61; H, 6.44; N, 9.42; S, 5.39. Found: C, 61.87; H, 6.259; N, 9.476; S, 5.519. HRMS *m/z* for C$_{31}$H$_{38}$N$_4$O$_6$S [M + H]$^+$ calcd. 595.3, found 595.3; [M + Na]$^+$ calcd. 617.2, found 617.3; [M + HCOOH]$^+$, calcd. 640.3, found 640.3; [M + Cl]$^-$, calcd. 629.2, found 629.3.

4.17. Benzyl ((S)-1-(((S)-3-methyl-1-oxo-1-((4-sulfamoylphenethyl)amino)butan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (*15*)

White solid (94%); mp 228–229 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.11 (t, 1H, N*H*CH$_2$CH$_2$, J = 6.0 Hz), 7.87 (d, 1H, OCONHCHCON*H*CH, J = 9.0 Hz), 7.74 (d, 2H, Ar—*H*, J = 6.0 Hz), 7.57 (d, 1H, OCON*H*CH, J = 9.0 Hz), 7.41–7.20 (m, 14H, Ar—*H* + N*H$_2$*), 4.95 (s, 2H, C*H$_2$*OCO), 4.38–4.30 (m, 1H, OCONHCHCONHC*H*), 4.12 (t, 1H, OCONHC*H*CON-HCH, J = 7.5 Hz), 3.40–3.28 (m, 2H, NHC*H$_2$*CH$_2$), 3.03–2.98 (m, 1H, CHC*H$_2$*Ph), 2.82–2.71 (m, 3H, CHC*H$_2$*Ph + NHCH$_2$C*H$_2$*), 1.92–1.86 (m, 1H, C*H*(CH$_3$)$_2$), 0.79 (d, 6H, CH(C*H$_3$*)$_2$, J = 6.0 Hz).

^{13}C NMR (300 MHz, DMSO-d_6): δ 171.3 (OCONHCHC*O*), 170.7 (*C*ONHCH$_2$CH$_2$), 155.8 (CH$_2$O*C*O), 143.5, 142.0, 138.0, 137.0, 129.2, 129.1, 128.2, 128.0, 127.6, 127.4, 126.2, 125.6 (Ar—*C*), 65.1 (*C*H$_2$OCO), 57.7 (OCONHCHCONH*C*H), 56.0 (OCONH*C*HCONHCH), 37.3 (CH*C*H$_2$Ph), 34.7 (CONHCH$_2$*C*H$_2$), 30.8 (*C*H(CH$_3$)$_2$), 19.1 and 18.0 (CH(*CH$_3$*)$_2$). ν(C=O)carbamate: 1639 cm^{-1}, ν(C=O)amide: 1689 cm^{-1}, ν(N−H)amine: 3299, 3676 cm^{-1}. Anal. calculated for $C_{30}H_{36}N_4O_6S$: C, 62.05; H, 6.25; N, 9.65; S, 5.52. Found: C, 61.89; H, 5.967; N, 9.673; S, 4.916. HRMS *m/z* for $C_{30}H_{36}N_4O_6S$ [M + H]$^+$ calcd. 581.2, found 581.2; [M + HCOOH]$^+$, calcd. 626.2, found 626.2; [M + Cl]$^-$, calcd. 615.2, found 615.2.

4.18. Benzyl ((S)-1-(((S)-4-(methylthio)-1-oxo-1-((4-sulfamoylphenethyl) amino)butan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**16**)

White solid (79%); mp 200–201 °C; ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 8.15 (d, 1H, OCONHCHCON*H*, *J* = 9.0 Hz), 7.97 (t, 1H, N*H*CH$_2$CH$_2$, *J* = 4.5 Hz), 7.76 (d, 2H, Ar−*H*, *J* = 9 Hz), 7.56 (d,1H, OCON*H*CH, *J* = 9.0 Hz), 7.41–7.19 (m, 14H, Ar−*H* + N*H*$_2$), 4.96 (s, 2H, C*H*$_2$OCO), 4.34–4.30 (m, 2H, OCONHC*H*CONHC*H*), 3.36–3.25 (m, 2H, NHC*H*$_2$CH$_2$), 3.06–3.00 (m, 1H, CHC*H*$_2$Ph), 2.81–2.72 (m, 3H, CHC*H*$_2$Ph + NHCH$_2$C*H*$_2$), 2.46–2.25 (m, 2H, CHCH$_2$C*H*$_2$SCH$_3$), 2.02 (s, 3H, CHCH$_2$CH$_2$SC*H*$_3$), 1.98–1.75 (m, 2H, CHC*H*$_2$CH$_2$SCH$_3$).

^{13}C NMR (300 MHz, DMSO-*d*$_6$): δ 171.4 (OCONHCH*C*O), 170.8 (*C*ONHCH$_2$CH$_2$), 155.9 (CH$_2$O*C*O), 143.6, 142.1, 138.0, 136.9, 129.2, 129.1, 128.3, 128.0, 127.7, 127.4, 126.2, 125.6 (Ar−*C*), 65.2 (*C*H$_2$OCO), 56.1 (OCONH*C*HCONHCH), 51.9 (OCONHCHCONH*C*H), 37.2 (CH*C*H$_2$Ph), 34.7 (NH*C*H$_2$CH$_2$), 32.0 (CHCH$_2$*C*H$_2$SCH$_3$), 29.5 (CHCH$_2$CH$_2$S*C*H$_3$), 14.6 CH*C*H$_2$CH$_2$SCH$_3$). ν(C=O)carbamate: 1643 cm^{-1}, ν(C=O)amide: 1697 cm^{-1}, ν(N−H)amine: 3277, 3371 cm^{-1}. Anal. calculated for $C_{30}H_{36}N_4O_6S_2$: C, 58.80; H, 5.92; N, 9.14; S, 10.46. Found: C, 58.18; H, 5.602; N, 8.978; S, 10.36. HRMS *m/z* for $C_{30}H_{36}N_4O_6S_2$ [M + H]$^+$ calcd. 613.2, found 613.2; [M + Na]$^+$, calcd. 635.2, found 635.2; [M + Cl]$^-$, calcd. 647.2, found 647.2.

4.19. Benzyl ((S)-4-methyl-1-oxo-1-(((S)-1-oxo-3-phenyl-1-((4-sulfamoylphenethyl)amino)propan-2-yl)amino)pentan-2-yl)carbamate (**17**)

White solid (95%); mp 109–110 °C; ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 8.11 (t, 1H, N*H*CH$_2$CH$_2$, *J* = 4.5 Hz), 7.94 (d, 1H, OCONHCHCON*H*, *J* = 9.0 Hz), 7.74 (d, 2H, Ar−*H*, *J* = 9.0 Hz), 7.44–7.17 (m, 15H, OCON*H*CH + Ar−*H* + N*H*$_2$), 5.04 (s, 2H, C*H*$_2$OCO), 4.50–4.43 (m, 1H, OCONHCHCONHC*H*), 4.04–3.96 (m, 1H, OCONHC*H*CONHCH), 3.35–3.25 (m, 2H, NHC*H*$_2$CH$_2$), 2.94–2.71 (m, 4H, CHC*H*$_2$Ph + NHCH$_2$C*H*$_2$), 1.51–1.28 (m, 3H, CHC*H*$_2$C*H*(CH$_3$)$_2$), 0.85–0.80 (m, 6H, CH(C*H*$_3$)$_2$). ^{13}C NMR (300 MHz, DMSO-*d*$_6$): δ 171.9 (OCONHCH*C*O), 170.7 (*C*ONHCH$_2$CH$_2$), 155.9 (CH$_2$O*C*O), 143.5, 142.0, 137.6, 137.0, 129.2, 129.1, 128.3, 128.0, 127.7, 127.6, 126.2, 125.6 (Ar−*C*), 65.4 (*C*H$_2$OCO), 53.7 (OCONHCHCONH*C*H), 53.3 (OCONH*C*HCONHCH), 40.7 (CH*C*H$_2$CH(CH$_3$)$_2$), 37.8 (CH*C*H$_2$Ph), 34.6 (CONH*C*H$_2$CH$_2$), 24.1 (CHCH$_2$*C*H(CH$_3$)$_2$), 22.9 and 21.4 (CHCH$_2$CH (*C*H$_3$)$_2$). ν(C=O)carbamate: 1642 cm^{-1}, ν(C=O)amide: 1676 cm^{-1}, ν(N−H)amine: 3302, 3676 cm^{-1}. Anal. calculated for $C_{31}H_{38}N_4O_6S$: C, 62.61; H, 6.44; N, 9.42; S, 5.39. Found: C, 61.92; H, 6.062; N, 9.258; S, 4.824. HRMS *m/z* for $C_{31}H_{38}N_4O_6S$ [M + H]$^+$ calcd. 595.3, found 595.3; [M + Na]$^+$, calcd. 617.2, found 617.3; [M + HCOOH]$^+$, calcd. 640.3, found 640.3; [M + Cl]$^-$, calcd. 629.2, found 629.2.

4.20. Benzyl (S)-(4-(methylthio)-1-oxo-1-((2-oxo-2-((4-sulfamoylphenethyl)amino)ethyl)amino)butan-2-yl)carbamate (**18**)

White solid (84%); mp 174–175 °C; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.21 (t, 1H N*H*CH$_2$CH$_2$, *J* = 6.0 Hz), 7.87 (t,1H, OCONHCHCON*H*, *J* = 6.0 Hz), 7.74 (d, 2H, Ar−*H*, *J* = 8.0 Hz), 7.62 (t, 1H, OCON*H*CH, *J* = 8.0 Hz), 7.40–7.30 (m, 9H, Ar−*H* + N*H*$_2$), 5.08–5.01 (m, 2H, C*H*$_2$OCO), 4.13–4.08 (m, 1H, OCONHC*H*CONHCH$_2$), 3.66 (d, 2H, C*H*$_2$CONHCH$_2$CH$_2$, *J* = 8.0 Hz), 3.32–3.30 (m, 2H, NHC*H*$_2$CH$_2$), 2.79 (t, 2H, NHCH$_2$C*H*$_2$, *J* = 6.0 Hz), 2.50–2.46 (m, 2H, CHCH$_2$C*H*$_2$SCH$_3$), 2.03 (s, 3H, CHCH$_2$CH$_2$SC*H*$_3$), 1.94–1.78 (m, 2H, CHC*H*$_2$CH$_2$SCH$_3$). ^{13}C NMR (400 MHz, DMSO-*d*$_6$): δ 172.3 (OCONHCH*C*O), 169.1 (*C*ONHCH$_2$CH$_2$), 156.7 (CH$_2$O*C*O), 144.0, 142.6, 137.4, 129.6, 128.8, 128.3, 128.2, 126.2 (Ar−*C*), 66.0 (*C*H$_2$OCO), 54.5 (OCONH*C*HCONHCH$_2$), 42.5 (OCONHCHCONH*C*H$_2$), 40.3 (CONH*C*H$_2$CH$_2$), 35.2 (CONHCH$_2$*C*H$_2$), 31.8 (CHCH$_2$*C*H$_2$SCH$_3$), 30.1 (CHCH$_2$CH$_2$S*C*H$_3$), 15.0 (CH*C*H$_2$CH$_2$SCH$_3$). ν(C=O)carbamate: 1654 cm^{-1}, ν(C=O)amide: 1689 cm^{-1}, ν(N−H)amine: 3299, 3413 cm^{-1}. Anal. calculated for $C_{23}H_{30}N_4O_6S_2$: C, 52.86; H, 5.79; N, 10.72; S, 12.27. Found: C, 51.91; H, 5.450; N, 10.40; S, 12.38. HRMS *m/z* for $C_{23}H_{30}N_4O_6S_2$ [M + H]$^+$ calcd. 523.2, found 523.1; [M + NH$_4$]$^+$, calcd. 540.2, found 540.2; [M + HCOOH]$^+$, calcd. 568.1, found 568.1; [M + Cl]$^-$, calcd. 557.1, found 557.1.

4.21. Benzyl ((S)-4-(methylthio)-1-oxo-1-(((S)-1-oxo-1-((4-sulfamoylphenethyl)amino)propan-2-yl)amino)butan-2-yl)carbamate (**19**)

White solid (88%); mp 235–236 °C; ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 8.01–7.99 (m, 2H, N*H*CH$_2$CH$_2$ + OCONHCHCON*H*), 7.75 (d, 2H, Ar−*H*, *J* = 9.0 Hz), 7.54 (d, 1H, OCON*H*CH, *J* = 9.0 Hz), 7.41–7.31 (m, 9H, Ar−H + NH$_2$), 5.04 (s, 2H, C*H*$_2$OCO), 4.25–4.07 (m, 2H, OCONHC*H*CONHC*H*), 3.37–3.23 (m, 2H, NHC*H*$_2$CH$_2$), 2.78 (t, 2H, NHCH$_2$C*H*$_2$, *J* = 7.5 Hz), 2.51–2.45 (m, 2H, CHCH$_2$C*H*$_2$SCH$_3$), 2.03 (s, 3H, CHCH$_2$CH$_2$SC*H*$_3$), 1.96–1.72 (m, 2H, CHC*H*$_2$CH$_2$SCH$_3$), 1.16 (d, 3H, C*H*$_3$, *J* = 6.0 Hz). ^{13}C NMR (300 MHz, DMSO-*d*$_6$): δ 172.0 (OCONHCH*C*O), 171.0 (*C*ONHCH$_2$CH$_2$), 156.0 (CH$_2$O*C*O), 143.6, 142.0, 137.0, 129.1, 128.3, 127.8, 127.6, 125.6 (Ar−*C*), 65.4 (*C*H$_2$OCO), 53.8 (OCONH*C*HCONHCH), 48.1 (OCONHCHCONH*C*H), 34.7 (NH*C*H$_2$CH$_2$), 31.7 (CHCH$_2$*C*H$_2$SCH$_3$), 29.6 (CHCH$_2$CH$_2$S*C*H$_3$), 18.4 (*C*H$_3$), 14.6 (CH*C*H$_2$CH$_2$SCH$_3$). ν(C=O)carbamate: 1636 cm^{-1}, ν(C=O)amide: 1688 cm^{-1}, ν(N−H)amine: 3309 cm^{-1}. Anal. calculated for $C_{24}H_{32}N_4O_6S_2$: C, 53.71; H, 6.01; N, 10.44; S, 11.95. Found: C, 53.22; H, 5.894; N, 9.771; S, 11.15. HRMS *m/z* for $C_{24}H_{32}N_4O_6S_2$ [M + H]$^+$ calcd. 537.2, found 537.2; [M + Na]$^+$, calcd. 559.2, found 559.2; [M + HCOOH]$^+$, calcd. 582.2, found 582.2; [M + Cl]$^-$, calcd. 671.1, found 571.1.

4.22. Benzyl((S)-4-(methylthio)-1-oxo-1-(((S)-1-oxo-3-phenyl-1-((4-sulfamoylphenethyl)amino)propan-2-yl)amino)butan-2-yl)carbamate (**20**)

White solid (94%); mp 216–217 °C; ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 8.12 (t, 1H, N*H*CH$_2$CH$_2$, *J* = 4.5 Hz), 8.01 (d, 1H, OCONHCHCON*H*, *J* = 9.0 Hz), 7.74 (d, 2H, Ar−*H*, *J* = 9.0 Hz), 7.52 (d, 1H, OCON*H*CH, *J* = 9.0 Hz), 7.37–7.18 (m, 14H, Ar−*H* + N*H*$_2$), 5.03 (s, 2H, C*H*$_2$OCO), 4.50–4.42 (m, 1H, OCONHCHCONHC*H*), 4.09–4.02 (m, 1H, OCONHC*H*CONHCH), 3.37–3.22 (m, 2H, NHC*H*$_2$CH$_2$), 2.95–2.71 (m, 4H, CHC*H*$_2$Ph + NHCH$_2$C*H*$_2$), 2.36 (t, 2H, CHCH$_2$C*H*$_2$SCH$_3$, *J* = 7.5 Hz), 2.00 (s, 3H, CHCH$_2$CH$_2$SC*H*$_3$), 1.79–1.70 (m, 2H, CHC*H*$_2$CH$_2$SCH$_3$). ^{13}C NMR (300 MHz, DMSO-*d*$_6$): δ 171.1 (OCONHCH*C*O), 170.7 (*C*ONHCH$_2$CH$_2$), 155.9 (CH$_2$O*C*O), 143.5, 142.0, 137.6, 136.9, 129.2, 129.1, 128.3, 128.0, 127.8, 127.7, 126.2, 125.6 (Ar−*C*), 65.5 (*C*H$_2$OCO), 54.0 (OCONHCHCONH*C*H), 53.8 (OCONH*C*HCONHCH), 37.7 (CH*C*H$_2$Ph), 34.7 (NHCH$_2$*C*H$_2$), 31.6 (CHCH$_2$*C*H$_2$SCH$_3$), 29.5 (CHCH$_2$CH$_2$S*C*H$_3$), 14.5 CH*C*H$_2$CH$_2$SCH$_3$). ν(C=O)carbamate: 1639 cm^{-1}, ν(C=O)amide: 1676 cm^{-1}, ν(N−H)amine: 3292 cm^{-1}. Anal. calculated for $C_{30}H_{36}N_4O_6S_2$: C, 58.80; H, 5.92; N, 9.14; S, 10.46. Found: C, 58.70; H, 5.554; N, 9.013; S, 10.95. HRMS *m/z* for $C_{30}H_{36}N_4O_6S_2$ [M + H]$^+$ calcd. 613.2, found 613.3; [M + Na]$^+$, calcd. 635.2, found 635.2; [M + HCOOH]$^+$, calcd. 658.2, found 658.3; [M + Cl]$^-$, calcd. 647.2, found 647.2.

4.23. Benzyl ((S)-4-(methylthio)-1-(((S)-4-(methylthio)-1-oxo-1-((4-sulfamoylphenethyl)amino)butan-2-yl)amino)-1-oxobutan-2-yl)carbamate (**21**)

White solid (95%); mp 199–200 °C; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.05–8.00 (m, 2H, N*H*CH$_2$CH$_2$ + OCONHCHCON*H*), 7.74 (d, 2H, Ar—*H*, *J* = 8.0 Hz), 7.55 (d,1H, OCON*H*CH, *J* = 8.0 Hz), 7.40–7.29 (m, 9H, Ar—H + $\mathrm{NH_2}$), 5.03 (s, 2H, $\mathrm{C}H_2\mathrm{OCO}$), 4.30–4.25 (m, 1H, OCONHCHCONHC*H*), 4.13–4.07 (m, 1H, OCONHC*H*CONHCH), 3.38–3.25 (m, 2H, $\mathrm{NHC}H_2\mathrm{CH_2}$), 2.78 (t, 2H, $\mathrm{NHCH_2C}H_2$, *J* = 6.0 Hz), 2.50–2.32 (m, 4H, $\mathrm{CHCH_2C}H_2\mathrm{SCH_3}$ + $\mathrm{CHCH_2C}H_2\mathrm{SCH_3}$), 2.03 (s, 3H, $\mathrm{CHCH_2CH_2SC}H_3$), 2.01 (s, 3H, $\mathrm{CHCH_2CH_2SC}H_3$), 1.93–1.74 (m, 4H, $\mathrm{CHC}H_2\mathrm{CH_2SCH_3}$ + $\mathrm{CHC}H_2\mathrm{CH_2SCH_3}$). ^{13}C NMR (400 MHz, DMSO-d_6): δ 171.9 (OCONHCHC*O*), 171.4 ($C\mathrm{ONHCH_2CH_2}$), 155.5 ($\mathrm{CH_2O}C\mathrm{O}$), 144.1, 142.6, 137.4, 129.6, 128.8, 128.3, 128.2, 126.1 (Ar—*C*), 65.9 ($C\mathrm{H_2OCO}$), 54.4 (OCONHCHCONH*C*H), 52.4 (OCONH*C*HCONHCH), 40.3 ($\mathrm{NH}C\mathrm{H_2CH_2}$), 35.2 ($\mathrm{NHCH_2}C\mathrm{H_2}$), 32.4 ($\mathrm{CHCH_2}C\mathrm{H_2SCH_3}$), 32.0 ($\mathrm{CH}C\mathrm{H_2CH_2SCH_3}$), 30.1 ($\mathrm{CHCH_2CH_2S}C\mathrm{H_3}$), 30.0 ($\mathrm{CHCH_2CH_2S}C\mathrm{H_3}$), 15.1 $\mathrm{CH}C\mathrm{H_2CH_2SCH_3}$ + $\mathrm{CH}C\mathrm{H_2CH_2SCH_3}$). ν(C=O)carbamate: 1628 cm^{-1}, ν(C=O)amide: 1653, 1693 cm^{-1}, ν(N—H)amine: 3308 cm^{-1}. Anal. calculated for $\mathrm{C_{26}H_{36}N_4O_6S_3}$: C, 52.33; H, 6.08; N, 9.39; S, 16.12. Found: C, 52.60; H, 5.841; N, 8.979; S, 16.13. HRMS *m/z* for $\mathrm{C_{26}H_{36}N_4O_6S_3}$ [M + H]$^+$ calcd. 597.2, found 597.2; [M + Na]$^+$, calcd. 619.2, found 619.2; [M + HCOOH]$^+$, calcd. 642.2, found 642.2; [M + Cl]$^-$, calcd. 631.1, found 631.1.

*4.24. tert-Butyl (S)-(1-oxo-1-((2-oxo-2-((4-sulfamoylphenethyl)amino)ethyl)amino)-3-phenylpropan-2-yl)carbamate (**22**)*

White solid (89%); mp 177–178 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.24 (t, 1H, $\mathrm{N}H\mathrm{CH_2CH_2}$, *J* = 6.0 Hz), 7.84 (t, 1H, OCONHCHCON*H*, *J* = 4.5 Hz), 7.76 (d, 2H, Ar—H, *J* = 9.0 Hz), 7.41 (d, 2H, Ar—*H*, *J* = 9.0 Hz), 7.31–7.18 (m, 7H, Ar—*H* + $\mathrm{N}H_2$), 7.06 (d, 1H, OCON*H*CH, *J* = 6.0 Hz), 4.21–4.13 (m, 1H, OCONHC*H*CO), 3.75–3.58 (m, 2H, $\mathrm{OCONHCHCONHC}H_2$), 3.35–3.28 (m, 2H, $\mathrm{NHC}H_2\mathrm{CH_2}$), 3.04–2.98 (m, 1H, $\mathrm{PhC}H_2\mathrm{CH}$), 2.83–2.71 (m, 3H, $\mathrm{PhC}H_2\mathrm{CH}$ + $\mathrm{NHCH_2C}H_2$), 1.31 (s, 9H, $\mathrm{OC(C}H_3)_3$). ^{13}C NMR (300 MHz, DMSO-d_6): δ 172.0 (OCONHCH-*C*O), 168.6 ($C\mathrm{ONHCH_2CH_2}$), 155.5 ($\mathrm{(CH_3)_3COC}O$), 143.5, 142.1, 138.1, 129.2, 129.1, 128.0, 126.1, 125.7 (Ar—*C*), 78.2 ($\mathrm{(CH_3)_3}C\mathrm{OCO}$), 55.9 ($\mathrm{OCONH}C\mathrm{HCONHCH_2}$), 42.1 ($\mathrm{OCONHCHCONH}C\mathrm{H_2}$), 37.2 ($\mathrm{CH}C\mathrm{H_2Ph}$), 34.8 ($\mathrm{CONHCH_2}C\mathrm{H_2}$), 28.1 ($\mathrm{(}C\mathrm{H_3)_3COCO}$). ν(C=O)carbamate: 1652 cm^{-1}, ν(C=O)amide: 1691 cm^{-1}, ν(N—H)amine: 3328, 3404 cm^{-1}. Anal. calculated for $\mathrm{C_{24}H_{32}N_4O_6S}$: C, 57.13; H, 6.39; N, 11.10; S, 6.35. Found: C, 56.93; H, 6.000; N, 11.07; S, 6.512. HRMS *m/z* for $\mathrm{C_{24}H_{32}N_4O_6S}$ [M + H]$^+$ calcd. 505.2, found 505.1; [M + Na]$^+$, calcd. 527.2, found 527.2; [M − H]$^-$, calcd. 503.2, found 503.1; [M + Cl]$^-$, calcd. 539.2, found 539.2; [M + HCOO]$^-$, calcd. 549.2, found 548.7.

*4.25. tert-Butyl ((S)-1-oxo-1-(((S)-1-oxo-3-phenyl-1-((4-sulfamoylphenethyl)amino)propan-2-yl)amino)-3-phenylpropan-2-yl)carbamate (**23**)*

White solid (95%); mp 205–206 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.18 (t, 1H, $\mathrm{N}H\mathrm{CH_2CH_2}$, *J* = 4.5 Hz), 8.10 (d, 1H, OCONHCHCON*H*, *J* = 6.0 Hz), 7.73 (d, 2H, Ar—*H*, *J* = 9.0 Hz), 7.35–7.16 (m, 15H, Ar—*H* + $\mathrm{N}H_2$), 6.96 (d, 1H, OCON*H*CH, *J* = 9.0 Hz), 4.51–4.44 (m, 1H, OCONHCHCONHC*H*), 4.16–4.09 (m, 1H, OCONHC*H*CONHCH), 3.35–3.21 (m, 2H, $\mathrm{NHC}H_2\mathrm{CH_2}$), 2.97–2.62 (m, 6H, $\mathrm{PhC}H_2\mathrm{CH}$ + $\mathrm{PhC}H_2\mathrm{CH}$ + $\mathrm{NHCH_2C}H_2$), 1.29 (s, 9H, $\mathrm{OC(C}H_3)_3$). ^{13}C NMR (300 MHz, DMSO-d_6): δ 171.2 (OCONHCHC*O*), 170.7 ($C\mathrm{ONHCH_2CH_2}$), 155.1 ($\mathrm{(CH_3)_3COC}O$), 143.3, 142.5, 138.0, 137.6, 129.2, 129.1, 129.0, 128.0, 127.9, 126.3, 126.1, 125.6 (Ar—*C*), 78.1 ($\mathrm{(CH_3)_3}C\mathrm{OCO}$), 55.9 (OCONHCHCONH*C*H), 53.9 (OCONH*C*HCONHCH), 38.0 ($\mathrm{CH}C\mathrm{H_2Ph}$), 37.5 ($\mathrm{CH}C\mathrm{H_2Ph}$), 34.6 ($\mathrm{CONHCH_2}C\mathrm{H_2}$), 28.1 ($\mathrm{(}C\mathrm{H_3)_3COCO}$). ν(C=O)carbamate: 1639 cm^{-1}, ν(C=O)amide: 1688 cm^{-1}, ν(N—H)amine: 3278 cm^{-1}. Anal. calculated for $\mathrm{C_{31}H_{38}N_4O_6S}$: C, 62.61; H, 6.44; N, 9.42; S, 5.39. Found: C, 61.93; H, 5.751; N, 8.962; S, 4.914. HRMS *m/z* for $\mathrm{C_{31}H_{38}N_4O_6S}$ [M + H]$^+$ calcd. 595.3, found 595.1; [M + Na]$^+$, calcd. 617.2, found 617.2; [M − H]$^-$, calcd. 593.2, found 593.2; [M + Cl]$^-$, calcd. 549.2, found 548.7; [M + HCOO]$^-$, calcd. 629.2, found 629.3.

*4.26. tert-Butyl ((S)-1-(((S)-4-methyl-1-oxo-1-((4-sulfamoylphenethyl)amino)pentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**24**)*

White solid (89%); mp 175–176 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.01 (t, 1H, $\mathrm{N}H\mathrm{CH_2CH_2}$, *J* = 4.0 Hz), 7.95 (d, 1H, OCONHCHCON*H*, *J* = 12.0 Hz), 7.73 (d, 2H, Ar—*H*, *J* = 8.0 Hz), 7.38 (d, 2H, Ar—*H*, *J* = 8.0 Hz), 7.26–7.17 (m, 7H, Ar—*H* + $\mathrm{N}H_2$), 6.98 (d, 1H, OCON*H*CH, *J* = 8.0 Hz), 4.29–4.14 (m, 2H, OCONHC*H*CONHC*H*), 3.35–3.23 (m, 2H, $\mathrm{NHC}H_2\mathrm{CH_2}$), 2.99–2.95 (m, 1H, $\mathrm{CHC}H_2\mathrm{Ph}$), 2.79–2.71 (m, 3H, $\mathrm{PhC}H_2\mathrm{CH}$ + $\mathrm{NHCH_2C}H_2$), 1.54–1.34 (m, 3H, $\mathrm{CHC}H_2\mathrm{C}H\mathrm{(CH_3)_2}$), 1.30 (s, 9H, $\mathrm{OC(C}H_3)_3$), 0.86–0.80 (m, 6H, $\mathrm{CH(C}H_3)_2$).

^{13}C NMR (400 MHz, DMSO-d_6): δ 172.3 (OCONHCHC*O*), 171.8 ($C\mathrm{ONHCH_2CH_2}$), 155.7 ($\mathrm{(CH_3)_3COC}O$), 144.0, 142.6, 138.6, 129.7, 129.6, 128.5, 126.6, 126.1 (Ar—*C*), 78.6 ($\mathrm{(CH_3)_3}C\mathrm{OCO}$), 56.2 (OCONH*C*HCONHCH), 51.5 (OCONHCHCONH*C*H), 41.8 ($\mathrm{CH}C\mathrm{H_2CH(CH_3)_2}$), 37.6 ($\mathrm{CH}C\mathrm{H_2Ph}$), 35.1 ($\mathrm{CONHCH_2}C\mathrm{H_2}$), 28.6 ($\mathrm{(}C\mathrm{H_3)_3COCO}$), 24.5 ($\mathrm{CHCH_2}C\mathrm{H(CH_3)_2}$), 23.4 and 22.2 ($\mathrm{CHCH_2CH(}C\mathrm{H_3)_2}$). ν(C=O)carbamate: 1644 cm^{-1}, ν(C=O)amide: 1689 cm^{-1}, ν(N—H)amine: 3322 cm^{-1}. Anal. calculated for $\mathrm{C_{28}H_{40}N_4O_6S}$: C, 59.98; H, 7.19; N, 9.99; S, 5.72. Found: C, 59.50; H, 6.801; N, 9.731; S, 5.592. HRMS *m/z* for $\mathrm{C_{28}H_{40}N_4O_6S}$ [M + H]$^+$ calcd. 561.3, found 561.2; [M + Na]$^+$, calcd. 585.3, found 583.2; [M − H]$^-$, calcd. 559.3, found 559.1; [M + Cl]$^-$, calcd. 595.2, found 595.3.

*4.27. tert-Butyl ((S)-1-(((2S,3S)-3-methyl-1-oxo-1-((4-sulfamoylphenethyl)amino)pentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**25**)*

White solid (88%); mp 200–201 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.02 (t, 1H, $\mathrm{N}H\mathrm{CH_2CH_2}$, *J* = 6.0 Hz), 7.96 (d, 1H, OCONHCHCON*H*, *J* = 9.0 Hz), 7.74 (d, 2H, Ar—*H*, *J* = 9.0 Hz), 7.38 (d, 2H, Ar—*H*, *J* = 6.0 Hz), 7.26–7.17 (m, 7H, Ar—*H* + $\mathrm{N}H_2$), 6.99 (d, 1H, OCON*H*CH, *J* = 9.0 Hz), 4.30–4.14 (m, 2H, OCONHC*H*CONHC*H*), 3.36–3.27 (m, 2H, $\mathrm{NHC}H_2\mathrm{CH_2}$), 2.99–2.94 (m, 1H, $\mathrm{CHC}H_2\mathrm{Ph}$), 2.79–2.64 (m, 3H, $\mathrm{PhC}H_2\mathrm{CH}$ + $\mathrm{NHCH_2C}H_2$), 1.53–1.36 (m, 3H, $\mathrm{C}H\mathrm{(CH_3)C}H_2\mathrm{CH_3}$), 1.30 (s, 9H, $\mathrm{OC(C}H_3)_3$), 0.87–0.80 (m, 6H, $\mathrm{CH(C}H_3\mathrm{)CH_2C}H_3$). ^{13}C NMR (300 MHz, DMSO-d_6): δ 172.3 (OCONHCHC*O*), 171.3 ($C\mathrm{ONHCH_2CH_2}$), 155.2 ($\mathrm{(CH_3)_3COC}O$), 143.4, 142.2, 138.1, 129.2, 129.1, 128.0, 126.1, 125.6 (Ar—*C*), 78.1 ($\mathrm{(CH_3)_3}C\mathrm{OCO}$), 55.7 (OCONHCHCONH*C*H), 51.0 (OCONH*C*HCONHCH), 41.3 ($\mathrm{CONH}C\mathrm{H_2CH_2}$), 37.10 ($\mathrm{CH}C\mathrm{H_2Ph}$), 34.6 ($\mathrm{CONHCH_2}C\mathrm{H_2}$), 28.1 ($\mathrm{(}C\mathrm{H_3)_3COCO}$), 24.0 ($C\mathrm{H(CH_3)CH_2CH_3}$), 22.9 ($\mathrm{CH(CH_3)}C\mathrm{H_2CH_3}$), 21.7 ($\mathrm{CH(}C\mathrm{H_3)CH_2}C\mathrm{H_3}$). ν(C=O)carbamate: 1645 cm^{-1}, ν(C=O)amide: 1692 cm^{-1}, ν(N—H)amine: 3315 cm^{-1}. Anal. calculated for $\mathrm{C_{28}H_{40}N_4O_6S}$: C, 59.98; H, 7.19; N, 9.99; S, 5.72. Found: C, 59.13; H, 6.857; N, 9.813; S, 5.741. HRMS *m/z* for $\mathrm{C_{28}H_{40}N_4O_6S}$ [M + H]$^+$ calcd. 561.3, found 561.2; [M + Na]$^+$, calcd. 585.3, found 583.2; [M − H]$^-$, calcd. 559.3, found 559.2; [M + Cl]$^-$, calcd. 595.2, found 595.2.

*4.28. tert-Butyl ((S)-1-(((S)-4-(methylthio)-1-oxo-1-((4-sulfamoylphenethyl)amino)butan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**26**)*

White solid (91%); mp 183–184 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.02 (d, 1H, OCONHCHCON*H*, *J* = 9.0 Hz), 7.96 (t, 1H, $\mathrm{N}H\mathrm{CH_2CH_2}$, *J* = 6.0 Hz), 7.75 (d, 2H, Ar—*H*, *J* = 9.0 Hz), 7.40 (d, 2H, Ar—*H*, *J* = 9.0 Hz), 7.27–7.17 (m, 7H, Ar—*H* + $\mathrm{N}H_2$), 7.04 (d, 1H, OCON*H*CH, *J* = 9.0 Hz), 4.34–4.14 (m, 2H, OCONHC*H*CONHC*H*), 3.37–3.24 (m, 2H, $\mathrm{NHC}H_2\mathrm{CH_2}$), 3.01–2.95 (m, 1H, $\mathrm{PhC}H_2\mathrm{CH}$), 2.78–2.71 (m, 3H, $\mathrm{PhC}H_2\mathrm{CH}$ + $\mathrm{NHCH_2C}H_2$), 2.43–2.35 (m, 2H, $\mathrm{CHCH_2C}H_2\mathrm{SCH_3}$), 2.02 (s, 3H, $\mathrm{CHCH_2CH_2SC}H_3$), 1.94–1.68 (m, 2H, $\mathrm{CHC}H_2\mathrm{CH_2SCH_3}$), 1.31 (s, 9H, $\mathrm{OC(C}H_3)_3$). ^{13}C NMR (300 MHz, DMSO-d_6): δ 171.6 (OCONHCHC*O*), 170.8 ($C\mathrm{ONHCH_2CH_2}$), 155.3 ($\mathrm{(CH_3)_3COC}O$), 143.5, 142.1, 138.1, 129.2, 129.1, 128.0, 126.2, 125.6 (Ar—*C*), 78.1 ($\mathrm{(CH_3)_3}C\mathrm{OCO}$), 55.8 (OCONH*C*HCONHCH), 51.8 (OCONHCHCONH*C*H), 37.0 ($\mathrm{CH}C\mathrm{H_2Ph}$), 34.6 ($\mathrm{CONHCH_2}C\mathrm{H_2}$), 32.1 ($\mathrm{CHCH_2}C\mathrm{H_2SCH_3}$), 29.4 ($\mathrm{CHCH_2CH_2S}C\mathrm{H_3}$),

28.1 ((CH$_3$)$_3$COCO), 14.6 (CH*C*H$_2$CH$_2$SCH$_3$). ν(C=O)carbamate: 1644 cm^{-1}, ν(C=O)amide: 1688 cm^{-1}, ν(N–H)amine: 3294 cm^{-1}. Anal. calculated for $C_{27}H_{38}N_4O_6S_2$: C, 56.03; H, 6.62; N, 9.68; S, 11.08. Found: C, 55.85; H, 6.331; N, 9.504; S, 11.28. HRMS *m/z* for $C_{27}H_{38}N_4O_6S_2$ [M + H]$^+$ calcd. 579.2, found 579.1; [M + Na]$^+$, calcd. 601.2, found 601.2; [M − H]$^-$, calcd. 577.2, found 577.2; [M + Cl]$^-$, calcd. 613.2, found 613.2.

4.29. General procedure for the synthesis of unprotected dipeptide-sulfonamide conjugates, 27–30

Boc-protected dipeptides (1.00 mmol) were dissolved in DCM/TFA (1:1) (5 mL) at room temperature and stirred for 1 h. The reaction mixture was evaporated, and the crude product obtained was diluted with 25 mL of saturated Na_2CO_3 and stirred for half an hour at room temperature. The desired product was than extracted by EtOAc (3x10 mL) and dried by anhydrous Na_2SO_4. After removing of Na_2SO_4 by filtration, filtrate was evaporated to give the corresponding unprotected crude dipeptide-sulfonamide conjugates. The crude product was than crystallized from EtOAc/*n*-hexane mixture (1:2).

4.30. (S)-2-((S)-2-amino-3-phenylpropanamido)-4-(methylthio)-N-(4-sulfamoylphenethyl)butanamide (27)

White solid (70%); mp 167–168 °C; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.03 (t, 2H, N*H*CHCON*H*, *J* = 6.0 Hz), 7.74 (d, 2H, Ar–*H*, *J* = 8.0 Hz), 7.39 (d, 2H, Ar–*H*, *J* = 8.0 Hz), 7.30–7.18 (m, 7H, Ar–*H* + N*H*$_2$), 4.31–4.30 (m, 1H, NH$_2$CHCONHC*H*), 3.48–3.26 (m, 3H, NH$_2$C*H*CONHCHCONHC*H*$_2$CH$_2$), 2.97–2.93 (m, 1H, PhC*H*$_2$CH), 2.79 (t, 2H, NHCH$_2$C*H*$_2$, *J* = 6.0 Hz), 2.70–2.65 (m, 1H, PhC*H*$_2$CH), 2.36–2.24 (m, 2H, CHCH$_2$C*H*$_2$SCH$_3$), 2.01 (s, 3H, CHCH$_2$CH$_2$SC*H*$_3$), 1.89–1.80 (m, 1H, CHC*H*$_2$CH$_2$SCH$_3$), 1.76–1.67 (m, 1H, CHC*H*$_2$CH$_2$SCH$_3$). ^{13}C NMR (400 MHz, DMSO-*d*$_6$): δ 174.7 (NH$_2$CH*C*O), 171.4 (*C*ONHCH$_2$CH$_2$), 144.0, 142.5, 139.0, 129.9, 129.6, 128.6, 126.6, 126.1 (Ar–*C*), 56.4 (NH$_2$*C*HCONHCH), 52.0 (NH$_2$CHCONH*C*H), 41.1 (CONH*C*H$_2$CH$_2$), 40.3 (CH*C*H$_2$Ph), 35.2 (CONHCH$_2$*C*H$_2$), 32.7 (CHCH$_2$*C*H$_2$SCH$_3$), 29.9 (CHCH$_2$CH$_2$S*C*H$_3$), 15.1 (CH*C*H$_2$CH$_2$SCH$_3$). ν(C=O)amide: 1648 cm^{-1}, ν(N–H)amine: 3289 cm^{-1}. Anal. calculated for $C_{22}H_{30}N_4O_4S_2$: C, 55.21; H, 6.32; N, 11.71; S, 13.40. Found: C, 54.87; H, 6.18; N, 11.56; S, 13.35. HRMS *m/z* for $C_{22}H_{30}N_4O_4S_2$ [M + H]$^+$ calcd. 478.2, found 479.2; [M − H]$^-$, calcd. 477.2, found 477.2; [M + Cl]$^-$, calcd. 513.2, found 513.2.

4.31. (2S,3S)-2-((S)-2-amino-3-phenylpropanamido)-3-methyl-N-(4-sulfamoylphenethyl)pentanamide (28)

White solid (73%); mp 182–183 °C; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.14 (t, 1H, N*H*CH$_2$CH$_2$, *J* = 4.0 Hz), 7.96 (d, 1H, NH$_2$CHCON*H*, *J* = 12.0 Hz), 7.74 (d, 2H, Ar–*H*, *J* = 8.0 Hz), 7.40 (d, 2H, Ar–*H*, *J* = 12.0 Hz), 7.30–7.18 (m, 6H, Ar–*H* + N*H*$_2$), 4.13 (t, 1H, NH$_2$CHCONHC*H*), 3.46–3.24 (m, 3H, NH$_2$C*H*CONHCHCONHC*H*$_2$CH$_2$), 3.01–2.96 (m, 1H, CHC*H*$_2$Ph), 2.79 (t, 2H, NHCH$_2$C*H*$_2$, *J* = 6.0 Hz), 2.67–2.62 (m, 1H, CHC*H*$_2$Ph), 1.74 (bs, 2H, N*H*$_2$CHCONH), 1.67–1.59 (m, 1H, C*H*(CH$_3$)CH$_2$CH$_3$), 1.36–1.28 (m, 1H, CH(CH$_3$)C*H*$_2$CH$_3$), 0.99–0.92 (m, 1H, CH(CH$_3$)C*H*$_2$CH$_3$), 0.79 (t, 3H, CH(CH$_3$)CH$_2$C*H*$_3$, *J* = 8.0 Hz), 0.73 (d, 3H, CH(C*H*$_3$)CH$_2$CH$_3$, *J* = 4.0 Hz). ^{13}C NMR (400 MHz, DMSO-*d*$_6$): δ 174.3 (NH$_2$CH*C*O), 171.3 (*C*ONHCH$_2$CH$_2$), 144.0, 142.6, 139.1, 129.9, 129.6, 128.6, 126.6, 126.1 (Ar–*C*), 56.9 (NH$_2$CHCONH*C*H), 56.4 (NH$_2$*C*HCONHCH), 41.1 (CONH*C*H$_2$CH$_2$), 40.1 (CH*C*H$_2$Ph), 37.5 (*C*H(CH$_3$)CH$_2$CH$_3$), 35.1 (CONHCH$_2$*C*H$_2$), 24.7 (CH(CH$_3$)*C*H$_2$CH$_3$), 15.7 (CH(*C*H$_3$)CH$_2$CH$_3$), 11.5 (CH(CH$_3$)CH$_2$*C*H$_3$). ν(C=O)amide: 1641, 1682 cm^{-1}, ν(N–H)amine: 3292, 3681 cm^{-1}. Anal. calculated for $C_{23}H_{32}N_4O_4S$: C, 59.98; H, 7.00; N, 12.16; S, 6.96. Found: C, 58.92; H, 7.01; N, 11.89; S, 6.92. HRMS *m/z* for $C_{23}H_{32}N_4O_4S$ [M + H]$^+$ calcd. 461.2, found 461.3; [M − H]$^-$, calcd. 459.2, found 459.3; [M + Cl]$^-$, calcd. 495.2, found 495.3; [M + HCOO]$^-$, calcd. 505.2, found 505.2.

4.32. (S)-2-((S)-2-amino-3-phenylpropanamido)-4-methyl-N-(4-sulfamoylbenzyl)pentanamide (29)

White solid (71%); mp 126–127 °C; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.63 (d, 1H, NH$_2$CHCON*H*, *J* = 8.0 Hz), 8.16 (t, 1H, N*H*CH$_2$CH$_2$ *J* = 4.0 Hz), 7.75 (d, 2H, Ar–*H*, *J* = 8.0 Hz), 7.40 (d, 2H, Ar–*H*, *J* = 8.0 Hz), 7.36–7.26 (m, 7H, Ar–*H* + N*H*$_2$), 4.32–4.26 (m, 1H, NH$_2$C*H*CONHCH), 4.08–4.05 (m, 1H, NH$_2$CHCONHC*H*), 3.13–3.08 (m, 1H, CHC*H*$_2$Ph), 2.97–2.92 (m, 1H, CHC*H*$_2$Ph), 2.80 (t, 2H, NHCH$_2$CH$_2$, *J* = 8.0 Hz), 1.59–1.38 (m, 3H, CHC*H*$_2$C*H*(CH$_3$)$_2$), 0.88–0.83 (m, 6H, CH(C*H*$_3$)$_2$). ^{13}C NMR (400 MHz, DMSO-*d*$_6$): δ 171.7 (NH$_2$CH*C*O), 168.2 (*C*ONHCH$_2$CH$_2$), 144.0, 142.6, 135.3, 130.0, 129.6, 128.9, 127.6, 126.1 (Ar–*C*), 53.7 (NH$_2$*C*HCONHCH), 51.8 (NH$_2$CHCONH*C*H), 41.8 (CH*C*H$_2$CH(CH$_3$)$_2$), 40.5 (CONH*C*H$_2$CH$_2$), 37.5 (CH*C*H$_2$Ph), 35.1 (CONHCH$_2$*C*H$_2$), 24.5 (CHCH$_2$*C*H(CH$_3$)$_2$), 23.3 and 22.3 (CHCH$_2$CH(*C*H$_3$)$_2$). ν(C=O)amide: 1658 cm^{-1}, ν(N–H)amine: 3266 cm^{-1}. Anal. calculated for $C_{23}H_{32}N_4O_4S$: C, 59.98; H, 7.00; N, 12.16; S, 6.96. Found: C, 59.45; H, 7.63; N, 12.33; S, 6.05. HRMS *m/z* for $C_{23}H_{32}N_4O_4S$ [M + H]$^+$ calcd. 460.2, found 461.3; [M − H]$^-$, calcd. 459.2, found 459.3.

4.33. (S)-2-amino-N-((S)-1-oxo-3-phenyl-1-((4-sulfamoylphenethyl)amino)propan-2-yl)-3-phenylpropanamide (30)

White solid (70%); mp 154–155 °C; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.10 (t, 1H, N*H*CH$_2$CH$_2$, *J* = 6.0 Hz), 8.05 (d, 1H, NH$_2$CHCON*H*, *J* = 4.0 Hz), 7.74 (d, 2H, Ar–*H*, *J* = 8.0 Hz), 7.36–7.12 (m, 14H, Ar–*H* + N*H*$_2$), 4.53–4.48 (m, 1H, NH$_2$C*H*CONHCH), 3.40–3.22 (m, 3H, NH$_2$CHCONHC*H*CONHCH$_2$C*H*$_2$), 2.89–2.72 (m, 5H, PhC*H*$_2$CH + PhC*H*$_2$CH + NHC*H*$_2$CH$_2$), 2.49–2.46 (m, 1H, NHC*H*$_2$CH$_2$). ^{13}C NMR (400 MHz, DMSO-*d*$_6$): δ 174.3 (NH$_2$CH*C*O), 171.2 (*C*ONHCH$_2$CH$_2$), 144.0, 142.6, 139.1, 137.9, 129.8, 129.8, 129.6, 128.6, 128.5, 126.8, 126.6, 126.2 (Ar–*C*), 56.5 (NH$_2$*C*HCONHCH), 53.8 (NH$_2$CHCONH*C*H), 40.1 (CONH*C*H$_2$CH$_2$), 38.6 (CH*C*H$_2$Ph), 37.5 (CH*C*H$_2$Ph), 35.2 (CONHCH$_2$*C*H$_2$). ν(C=O)amide: 1638 cm^{-1}, ν(N–H)amine: 3317 cm^{-1}. Anal. calculated for $C_{26}H_{30}N_4O_4S$: C, 63.14; H, 6.11; N, 11.33; S, 6.48. Found: C, 63.45; H, 6.00; N, 11.10; S, 6.27. HRMS *m/z* for $C_{26}H_{30}N_4O_4S$ [M + H]$^+$ calcd. 495.2, found 495.2; [M − H]$^-$, calcd. 493.2, found 493.3; [M + Cl]$^-$, calcd. 529.2, found 529.2; [M + HCOO]$^-$, calcd. 539.2, found 539.2.

4.34. CA inhibition

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity by using method of Khalifah [29]. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM HEPES (pH 7.5) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5%–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled–deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-square methods using PRISM (www.graphpad.com), and non-linear least squares methods, values representing the mean of at least three different determinations, as described earlier by us [35–41].

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Declaration of interest

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.11.003.

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