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Regioselective Synthesis of a Family of β-Lactams Bearing a Triazole Moiety as Potential Apoptosis Inhibitors

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Apoptosis is a biological process important to several human diseases; it is strongly regulated through protein–protein interactions and complex formation. We previously reported the synthesis of apoptosis inhibitors bearing an exocyclic triazole amide isoster by using an Ugi four-component coupling reaction (Ugi-4CC), followed by a base-promoted intramolecular cyclization. Depending on the substitution patterns and the reaction conditions, this cyclization forms the six- or four-membered ring. Two compounds bearing the β-lactam scaffold turned out to be the most potent inhibitors. This encouraged us to optimize the modulation of the cyclization, and prepare a library of 15 β-lactams with total regioselectivity. Moreover, we aimed to improve the bioavailability of these compounds through the introduction of diversity at different substitution positions. The activity of these compounds as apoptosis inhibitors in cellular extracts has been evaluated, showing an increase in their potency.

1. Introduction

The process of programmed cell death, known as apoptosis, is a highly regulated cellular pathway that plays a central role in development, normal cell turnover, and immune system function.[1] Anomalies in apoptosis (either inappropriate suppression or induction) lead to severe health problems, such as cancer pathogenesis,[2] neurodegenerative, heart diseases,[3,4] among others.

In a signaling cascade, initiator caspases (e.g. caspases-8, -9, and -10) activate effector caspases (e.g. caspases-3 and -7), which are responsible for the disassembly of cellular components.[5] The mitochondria-mediated pathway is activated by intracellular signals and uses caspase-9 as the initiator.[6] The formation of the macromolecular complex named apoptosome is a key event in this intrinsic pathway. The apoptosome is a holoenzyme multiprotein complex formed from cytochrome *c*, activated Apaf-1 (apoptosis protease-activating factor 1), dATP, and procaspase-9.[7] Knowledge of the functional activation mechanism of the apoptosome has helped the definition of prospective targets for treating deregulated apoptosis associated with human pathologies.[8] Along these lines, inactivation of the apoptosome might be a therapeutic strategy for treating not only neurodegenerative, but other organ dysfunctions such as ischemia, cardiac, and renal failure.[3,4,9] In addition, it can be a useful tool for the preservation of organs for transplant. Thus, Apaf-1 should be considered as an attractive target for the development of apoptosis modulators.

We previously reported the synthesis of conformationally restricted apoptosis inhibitors bearing an exocyclic triazole moiety by using an Ugi four-component coupling reaction (Ugi-4CC), followed by a base-promoted intramolecular cyclization.[10] The cyclization led to the corresponding six-membered ring (2,5-diketopiperazine, DKP) or four-membered ring, as the intramolecular alkylation reaction can occur either through the nitrogen or the α-carbon atom of the secondary amide formed during the Ugi-4CC (Scheme 1 A). The ratio between the DKP and the β-lactam products depended on the relative acidity of the NH and α-CH hydrogen atoms, which were clearly affected by the substitution pattern in the triazole ring or in the amide nitrogen atom. The nature of the base and the solvent was also crucial in this regard.

Biological evaluation of a preliminary collection of conformationally constrained cyclic compounds bearing an exocyclic 2,4-triazole moiety, revealed compounds **1** and **2a**, both bearing the β-lactam scaffold, as the most potent apoptosis inhibitors tested in an in vitro assay (Scheme 1 B).[10] Moreover, two essential substituents for the activity were identified: diphenylpropyl at R^3 and 2,4-dichlorophenetyl at R^1. However, **1** and **2a** showed to be highly hydrophobic molecules, which could lead to undesired side effects, unspecific toxicity, and non-optimal pharmacokinetics in further stages of drug development. With

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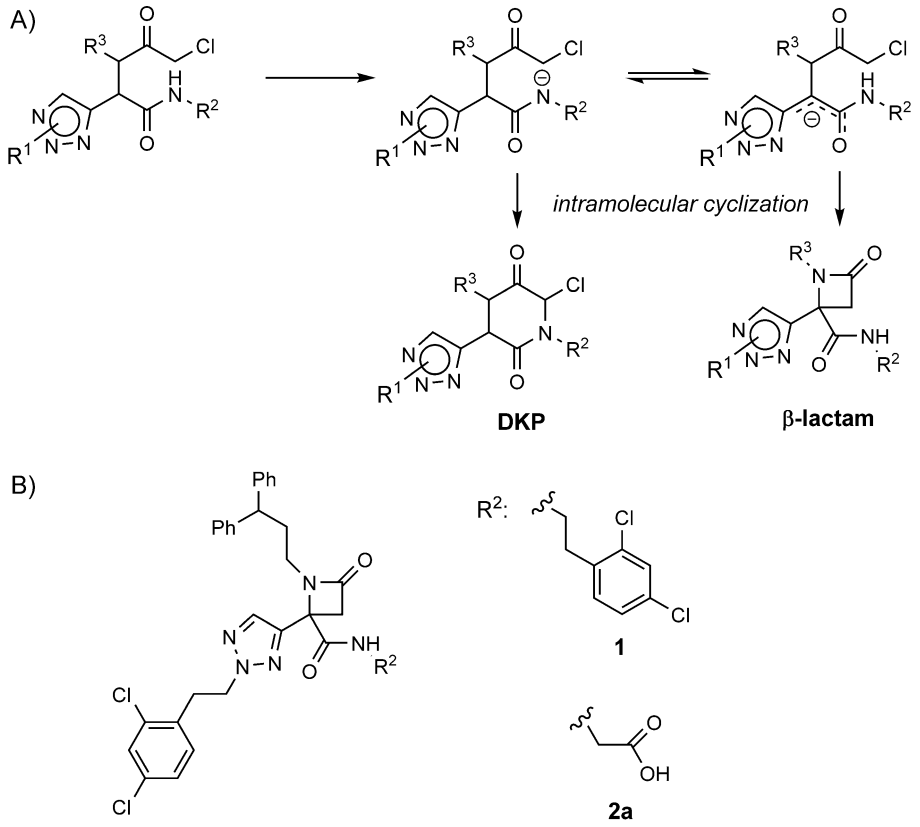


Scheme 1. A) Intramolecular cyclization leading to the corresponding six- (DKP) or four-membered ring (β-lactam), depending on the reaction conditions and the substitution pattern at R^1 and R^2. B) Compounds **1** and **2a** are the most potent apoptosis inhibitors tested in an in vitro assay.

these antecedents, we report an optimized synthesis of a library of β-lactams bearing the 2,4-dichlorophenetyl moiety at R^1, which took place with total regioselectivity. Moreover, we envisioned the introduction of different substituents at R^3 to enhance the inhibitory potency of these compounds. We also introduced variability at R^2 with the aim of improving the bioavailability of the new synthesized apoptosis inhibitors.

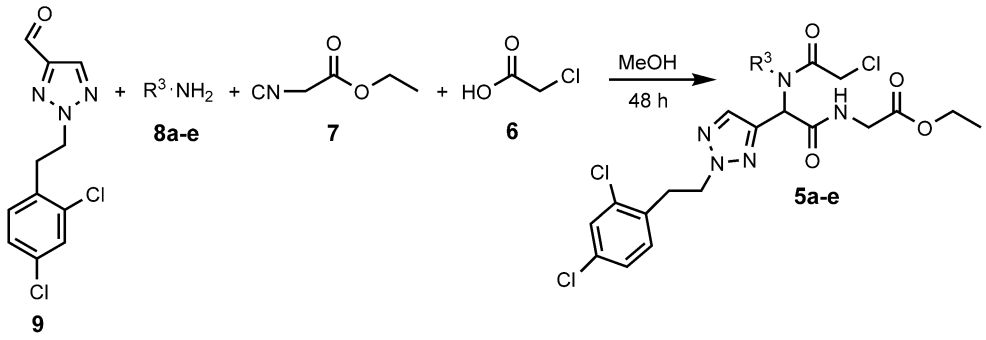
2. Results and Discussion

2.1. Regioselective Synthesis of β-Lactams 3

The most common methods used for the synthesis of β-lactams involve ketene–imine cyclizations[11] (the Staudinger reaction) and ester enolate–imine condensations[12] (the Gilman–Speeter reaction). Other approaches such as photoinduced rearrangements[13] and radical cyclizations[14] have also been employed. A less common procedure for the synthesis of these compounds is the Ugi-4CC. Fülop and co-workers described the liquid- and solid-phase combinatorial synthesis of bicyclic β-lactams from monocyclic β-amino acids through Ugi-4CC.[15,16] Besides bicyclic systems, Ugi- 4CC addressed to prepare monocyclic β-lactams are also described. Pirrung and Das Sarma reported the preparation of a 32 β-lactam library in water in good yields, but without diastereoselectivity.[17] Likewise, the Pepino group published a facile two-step synthesis of β-lactams based on the reaction between (*E*)-cinnamaldehyde, chloroacetic acid, cyclohexyl isocyanide, and primary amines to afford the expected Ugi adduct. The final ring-closure reaction gave the corresponding β-lactams in high yields.[18]

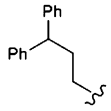
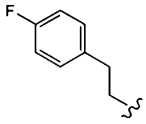
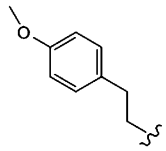
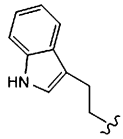
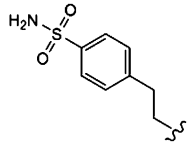
In the present work, we took advantage of the Ugi-4CC approach and the versatility of the subsequent intramolecular cyclization, which affords two different compounds (six- or four-membered rings) depending on the reaction conditions and the substitution patterns, to prepare a library of 15 β-lactam derivatives with total regioselectivity. Considering that the 2,4-dichlorophenetyl moiety at R^1 was crucial for the biological activity,[10] we also envisioned the introduction of different substituents at R^2 and R^3 with the aim of enhancing the bioavailability and, therefore, the activity of these β-lactams as apoptosis inhibitors.

The synthetic approach for obtaining the 15 β-lactam library started with an Ugi-4CC, which involved carboxylic acid **6**, isocyanide **7**, primary amines **8a–e**, and aldehyde **9** to yield the corresponding Ugi adducts **5a–e** (Scheme 2). Most of the starting materials (**6**, **7**, and **8a–e**) are commercially available. However, the triazole aldehyde **9** bearing the 2,4-dichlorophenetyl moiety had to be prepared as previously reported.[19] No substantial differences in the reaction yields were observed when performing the Ugi-4CC, obtaining values from 60 to 76% with the different amines (Table 1). This step is essential to intro-

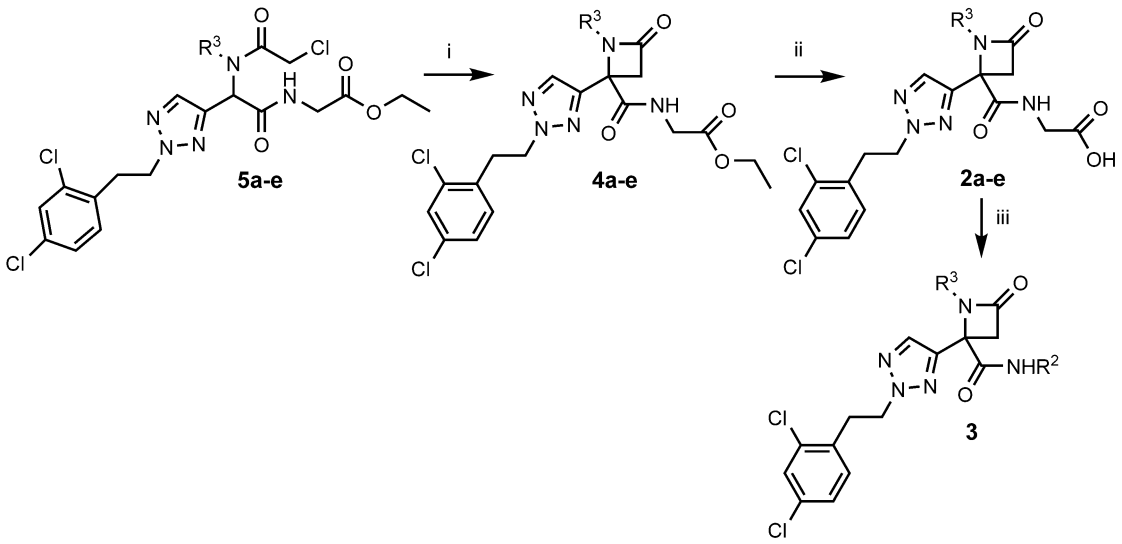


Scheme 2. Synthesis of Ugi adducts **5a–e**.

Table 1. Synthesis of Ugi adducts by using amines **8a–e**.

Entry	Compound	R^3	Yield [%]
1	**5a**[12]		70
2	**5b**		60
3	**5c**		76
4	**5d**		63
5	**5e**		76

duce variability at R^3 in the library of β-lactams, and the robustness of the reaction was highly useful. The Ugi-4CC requires a high concentration in the mixture and a specific order of addition. From our previous work,[19] we established that the imine formation is completed after 6 h and, afterward, the addition of the isocyanide and the carboxylic acid must be done within 30 min to avoid imine hydrolysis. The key reaction for β-lactam ring formation was a base-promoted intramolecular cyclization (Scheme 3). As previously reported,[3] this reaction can lead to the six- or four-membered ring, depending on different factors such as the substitution pattern in the triazole ring, the nature of the substituent at R^2, and the reaction conditions (base and solvent). In our case, treatment of Ugi adducts **5a–e** with DBU/THF afforded β-lactams **4a–e** in very good yields (81–99%) with total regioselectivity. That is, no DKP formation was observed in any case, as confirmed by the absence of the CH of the chiral center of the DKP signal at around 5.1 ppm from the ^{1}H NMR spectra, in addition to the presence of the amide NH signal. Moreover, new signals appeared in the ^{1}H NMR spectra, which are characteristic for the diastereotopic protons of the β-lactam ring: two doublets at around 3.5 and 3.4 ppm. The total regioselectivity to the β-lactam ring achieved during the cyclization is attributed to the presence of an ethyl ester group at R^2 that reduces the amide acidity and, consequently, the possibility of concomitant DKP formation. Likewise, the low polarity of the solvent and the low hardness of the base favor the β-lactam ring. Subsequent saponification of the ethyl ester under basic conditions afforded the corresponding carboxylic acids **2a–e** (yields from 66 to 94%) (Table 2), which were accessible for further introduction of diversity at R^2. Finally, coupling reactions with 2-methoxyethanamine and *N*-(2-*a*minoethyl)acetamide, using EDC/HOBt, yielded the diverse library of β-lactams **3aA**, **3aB**, **3bA**, **3bB**, **3cA**, **3cB**, **3dA**, **3dB**, and **3eA**, **3eB** in yields from 23 to 63% (Table 2). The hydrophilic character of the new β-lactams compared to compounds **1** and **2a** was estimated from the retention time in reverse-phase HPLC analysis (Table 2). Thus, the introduction of different substituents at R^3 conferred an increased polarity to the β-lactams in the order: 4-fluorophenethyl < 4-methoxyphenethyl < triptaminyl < 4-(2-ethyl)benzenesulfonamidyl. In this way, compounds **2a**, **3aA**, and **3aB** bearing the diphenylpropyl moiety at R^3 were the least polar (entries 2, 7, and 8, Table 2). On the other hand, compounds **2e**, **3eA**, and **3eB** with 4-(2-ethyl)benzenesulfonamidyl at R^3 were the most polar (entries 6, 15, and 16, Table 2). Comparing families of compounds with the same substituent at R^3, the introduction of (2-acetamidylethyl)aminocarbonylmethyl at R^2 provided a higher hydrophilicity than the (2-methoxyethyl)-aminocarbonylmethyl group. Thus, β-lactam **3eB** bearing 4-(2-ethyl)benzenesulfonamidyl at R^3 and (2-acetamidylethyl)aminocarbonylmethyl at R^2 was the most polar compound of the library (entry 16, Table 2). As polarity is an important physicochemical parameter for drug bioavailability, the modular implementation of different residues conferring a relatively wide range of polarity to the final molecule must be considered an advantage for further development of the chemical entities.



Scheme 3. Synthesis of the β-lactams through intramolecular cyclization, followed by basic hydrolysis and coupling reactions with different amines: i) DBU, THF, 20 °C, 18 h, 70–91 % yield; ii) LiOH, 1:1 THF/H_2O, 20 °C, 6 h, 66–94 % yield; iii) R_2NH_2, EDC, HOBt, DIPEA, CH_2Cl_2, 20 °C, 18 h, 23–63 % yield.

2.2. Biological Activity of β-Lactams 3

The new β-lactams were tested to determine their capacity to prevent the activation of caspase-3. As this caspase acts directly on specific cellular substrates to dismantle the cell, the decrease of its activity results in the inhibition of apoptosis.

The apoptosome reconstitution assay was carried out by incubating HEK-293 cell extracts depleted of Apaf-1 (containing caspase-3, caspase-9, and cytochrome *c*), rApaf-1, and dATP in the presence of tested compounds. After addition of caspase-3 fluorogenic substrate Ac-DEVD-afc, which is enzymatically cleaved by activated caspase-3, the caspase activity was continuously monitored by measuring the release of the afc group.[20] At 25 μM, β-lactams **3aA**, **3aB**, **2b**, **3cA**, **2d**, and **3dA** displayed a caspase-3 inhibition higher than 80 % (Figure 1 A). Moreover, these compounds were more potent than the original β-lactam **1** and comparable to **2a** at this concentration. At a lower concentration (12.5 μM), β-lactams **3aA**, **3aB**, **3bA**, **3bB**, **3cA**, and **3cB** showed an inhibitory activity higher than 60 %, as shown in Figure 1 B. In this case, **3aA** and **3aB**, with an inhibition higher than 85 %, were even better inhibitors than the original compound **2a**. Surprisingly, compounds **3bA**, **3dB**, **2c**, and **2e** displayed a higher activity at a lower concentration. This might be attributed to the tendency of self-aggregation of these compounds at high concentrations. As β-lactam **3aA** was the best inhibitor of the library, we also determined its IC_{50} value (concentration at which the response is reduced by half) in this assay (7.3 ± 4.1 μM).

Based on these results, we can conclude that the diphenylpropyl, 4-methoxyphenetyl, and 4-fluorophenetyl substituents at R^3 displayed the highest inhibitory activities. On the other hand, (2-methoxyethyl)aminocarbonylmethyl and (2-acetamidylethyl) aminocarbonylmethyl at R^2 were important for the activity, as β-lactams bearing these substituents were more active than the corresponding free acids. The best inhibitors of the 15 β-lactam library were **3aA** and **3aB**, which means that the diphenylpropyl group at R^3 is crucial for the biological activity. These trends were in agreement with previous results obtained with other generations of apoptosis inhibitors.[10] Noticeably, these two new derivatives **3aA** and **3aB** displayed an improved aqueous solubility, with the concomitant drugability advantages.

3. Conclusions

We report the regioselective synthesis of a diverse library of β-lactams bearing a 2,4-triazole based on an Ugi-4CC, followed by a base-promoted intramolecular cyclization. The Ugi-4CC

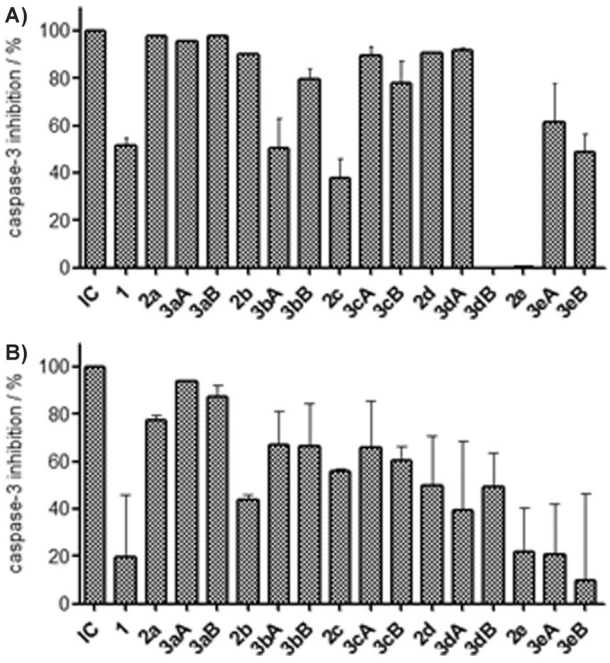
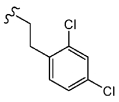
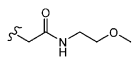
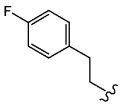
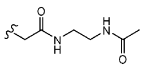
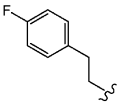
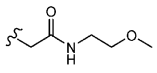
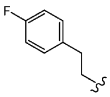
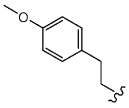
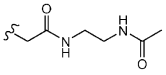
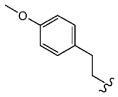
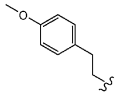
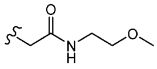
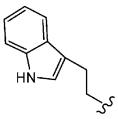
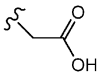
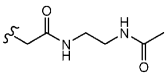
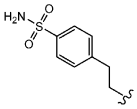
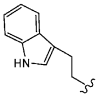
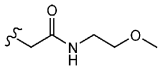
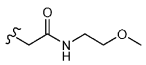
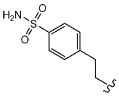
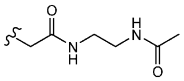
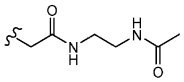
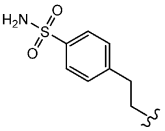


Figure 1. Caspase-3 inhibition in HEK-293 cell extracts. Cell extracts were exposed to A) 25 μM and B) 12.5 μM of compounds **1** and **2a** and the library of β-lactams. Values are given as mean ± SD, $n = 2$.

Table 2. Synthesis of the 15 β-lactams library and their retention times determined in reverse-phase HPLC (see Experimental Section for conditions).

Entry	β-Lactam	R^2	R^3	Yield [%]	Time [min]
1	**1**				18.90
2	**2a**			71[a]	13.58
3	**2b**			64[a]	12.02
4	**2c**			57[a]	11.80
5	**2d**			66[a]	11.56
6	**2e**			46[a]	9.78
7	**3aA**			63[b]	13.67
8	**3aB**			42[b]	12.54
9	**3bA**			53[b]	12.05
10	**3bB**			53[b]	10.97
11	**3cA**			57[b]	11.75
12	**3cB**			53[b]	10.73
13	**3dA**			30[b]	11.55
14	**3dB**			24[b]	10.54
15	**3eA**			31[b]	9.78
16	**3eB**			23[b]	9.00

[a] Overall yield of cyclization and ester hydrolysis. [b] Single yield of coupling reactions.

with different amines allowed us to introduce diversity at R^3. The key step for the formation of the four-membered ring was the intramolecular cyclization, using DBU as non-nucleophilic base in THF. Under these reaction conditions, no formation of the alternative six-membered ring was observed, providing total regioselectivity towards the β-lactam scaffold. After ethyl ester hydrolysis, further coupling reactions also provided the introduction of different substitution patterns at R^2. The diverse library of 15 β-lactams displayed higher hydrophilic character than the previously reported compounds.

The newly synthesized β-lactams were tested as caspase-3 inhibitors (apoptosis inhibitors) in HEK-293 cell extracts at two different concentrations. Results obtained from this assay showed interesting trends. First of all, the diphenylpropyl, 4-methoxyphenetyl, and 4-fluorophenetyl substituents at R^3 enhanced the inhibitory activity. Moreover, (2-methoxyethyl)aminocarbonylmethyl and (2-acetamidylethyl)aminocarbonylmethyl at R^2 also improved the activity of the compounds, giving higher values of inhibition. Thus, we demonstrated that by introducing variability at R^2 to improve the physicochemical properties, the activity of our compounds has been also potentiated.

Experimental Section

General

Chemicals were obtained from commercial sources and used without further purification. Anhydrous solvents were obtained from PureSolv M (Solvent Purification System). ^{1}H NMR spectra were recorded in a Varian Unity Inova 500 or VMRS 400 spectrometer. ^{13}C NMR spectra were recorded in a VMRS 400 spectrometer. Chemical shifts (δ) are reported in ppm relative to the solvent ($CDCl_3$ or CD_3OD). All reactions were monitored by using TLC on aluminum foil pre-coated silica gel plates. Flash column chromatography was performed with the indicated solvents by using silica gel 60 (particle size 35–70 μm). Preparative RP chromatography was performed with a Biotage system, using a KP-C18-HS 12 g column and the indicated solvents. Preparative TLC was performed by using a silica gel 60 glass plate (F_{254}, 0.5 mm) and the indicated solvents. Analytical RP-HPLC was performed with a Hewlett Packard Series 110 (UV detector 1315A) modular system, using a RP Kromasil 100 C18 (15×0.46 cm, 5 μm) column. CH_3CN/H_2O mixtures containing 0.1% TFA at 1 mL min^{-1} were used as mobile phase, and the monitoring wavelength was set at 220 or 254 nm. A gradient method from 20 to 100% CH_3CN in 20 min was used. High-resolution mass spectra were obtained with a LCT premier UPLC/MS Q-TOF (Waters) and an electrospray ionization detector.

Synthesis of Ugi Adducts 5a–e

General Procedure

To a solution of aldehyde **9** (0.40 mmol) in MeOH (200 μL), the corresponding amine (0.40 mmol) was added. The resulting mixture was stirred for 6 h at room temperature, monitoring the imine formation by ^{1}H NMR spectroscopy. Next, a solution of ethyl isocyanoacetate (0.40 mmol) in MeOH (100 μL) was added, followed by a solution of chloroacetic acid (0.40 mmol) in MeOH (100 μL). The resulting mixture was stirred for 48 h at room temperature, and

concentrated in vacuo to afford crude compounds, which were purified as indicated.

Ugi Adduct 5a

210 mg (0.31 mmol, 70%) of **5a** were obtained from 3,3-diphenylpropylamine (80 μL, 0.39 mmol) as a white solid. Purification was accomplished through flash chromatography (1:0 to 1:1 hexane/EtOAc gradient). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.76 (s, 1H, H11), 7.35 (d, J = 2 Hz, 1H, H14), 7.30–7.27 (m, 4H, H16), 7.21–7.16 (m, 6H, H15, 17), 7.05 (dd, J = 8.2, 2 Hz, 1H, H13), 6.9 (m, 1H, NH), 6.89 (d, J = 8.2 Hz, 1H, H12), 5.54 (s, 1H, H1), 4.63 (t, J = 7.1 Hz, 2H, H6), 4.19 (q, J = 7.1 Hz, H9), 4.09 (dd, J = 18, 5 Hz, 1H, H8), 3.97 (dd, J = 18, 5 Hz, 1H, H8), 3.83 (t, J = 8 Hz, 1H, H3), 3.77 (s, 2H, H2), 3.3 (m, 4H, H7, H5), 2.3 (m, 1H, H4), 2.2 (m, 1H, H4), 1.27 ppm (t, J = 7.1 Hz, 3H, H10). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.3 (C25), 167.3 (C24), 167.2 (C23), 143.3 (C22), 143.2 (C22), 142.4 (C18), 134.9 (C11), 134.8 (C20), 133.5 (2C, C19,21), 131.6 (C12), 129.5 (C14), 128.8 (2C, C16), 128.7 (2C, C16), 127.6 (2C, C15), 127.5 (2C, C15), 127.2 (C13), 126.8 (C17), 126.7 (C17), 61.5 (C9), 56.9 (C1), 54.0 (C6), 48.4 (C3), 47.4 (C7), 41.6 (C8), 40.9 (C2), 34.8 (C4), 33.5 (C5), 14.1 ppm (C10). HRMS calculated for $C_{33}H_{35}Cl_3N_5O_4$: 670.1755 $[M+H]^+$. Found: 670.1794. Calculated: HRMS calculated for $C_{33}H_{34}Cl_3N_5NaO_4$: 693.1652 $[M+Na]^+$. Found: 693.1608.

Ugi Adduct 5b

181 mg (0.30 mmol, 60%) of **5b** were obtained from 4-fluorophenethylamine (66 μL, 0.50 mmol) as a white solid. Purification was accomplished through flash chromatography (1:0 to 1:1 hexane/EtOAc gradient). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.84 (s, 1H, H10), 7.32 (d, J = 2 Hz, 1H, H13), 7.12–7.05 (m, 2H, HAr), 7.04–6.91 (m, 4H, HAr), 6.89–6.84 (m, 1H, NH), 5.66 (s, 1H, H1), 4.70 (t, J = 7 Hz, 2H, H5), 4.21 (q, J = 7 Hz, 2H, H8), 4.10 (dd, J = 18, 5 Hz, 1H, H7), 4.05 (dd, J = 18, 5 Hz, 1H, H7), 3.87 (s, 2H, H2), 3.60–3.47 (m, 2H, H4), 3.37 (t, J = 7 Hz, 2H, H6), 2.82–2.73 (m, 1H, H3), 2.68–2.59 (m, 1H, H3), 1.28 ppm (t, J = 7 Hz, 3H, H9). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.5 (C24), 167.5 (C23), 167.4 (C22), 162.0 (d, $J_{C\text{-}F}$ = 245 Hz, C21), 142.4 (C16), 135.3 (C10), 134.9 (C18), 133.7 (d, $J_{C\text{-}F}$ = 10 Hz, C20), 133.5 (C19), 133.4 (C17), 131.8 (C12), 130.4 (d, $J_{C\text{-}F}$ = 7 Hz, C14) 129.7 (C13), 127.4 (C11), 115.9 (d, $J_{C\text{-}F}$ = 22 Hz, C15), 61.8 (C8), 57.1 (C1), 54.2 (C5), 50.6 (C4), 41.8 (C7), 41.1 (C2), 34.8 (C3), 33.6 (C6), 14.3 ppm (C9). HRMS calculated for $C_{26}H_{27}Cl_3FN_5NaO_4$: 620.1010 $[M+Na]^+$. Found: 620.1013.

Ugi Adduct 5c

250 mg (0.41 mmol, 76%) of **5c** were obtained from 4-methoxyphenethylamine (79 μL, 0.54 mmol) as a colorless oil. Purification was accomplished through flash chromatography (1:0 to 1:1 hexane/EtOAc gradient). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.85 (s, 1H, H10), 7.33 (d, J = 2 Hz, 1H, H13), 7.04–7.00 (m, 3H, H12, H14), 6.94 (d, J = 8 Hz, 1H, H11), 6.90–6.85 (m, 1H, NH), 6.85–6.80 (m, 2H, H15), 5.66 (s, 1H, H1), 4.70 (t, J = 7 Hz, 2H, H5), 4.21 (q, J = 7 Hz, 2H, H8), 4.10 (dd, J = 18, 6 Hz, 1H, H7), 4.00 (dd, J = 18, 5 Hz, 1H, H7), 3.84 (s, 2H, H2), 3.78 (s, 3H, H16), 3.59–3.47 (m, 2H, H4), 3.37 (t, J = 7 Hz, 2H, H6), 2.77–2.67 (m, 1H, H3), 2.65–2.56 (m, 1H, H3), 1.27 ppm (t, J = 7 Hz, 3H, H9). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.5 (C25), 167.5 (CO), 167.4 (CO), 158.7 (C22), 142.5 (C17), 135.3 (C10), 134.9 (C19), 133.7 (C20), 133.6 (C18), 131.8 (C11), 129.9 (C14, C13), 129.6 (C12), 127.4 (C14), 114.4 (C15), 61.7 (C8), 57.2 (C1), 55.4 (C16), 54.2 (C5), 50.8 (C2), 41.9 (C7), 41.1 (C2), 34.7 (C3), 33.6 (C6), 14.3 ppm (C9). HRMS calculated for $C_{27}H_{30}Cl_3N_5NaO_5$: 632.1210 $[M+Na]^+$. Found: 632.1215.

Ugi Adduct 5d

242 mg (0.39 mmol, 63%) of **5d** were obtained from tryptamine (99 mg, 0.62 mmol) as a yellow oil. Purification was accomplished through flash chromatography (10:0 to 4:6 hexane/EtOAc gradient).^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.10 (brs, 1H, NH), 7.85 (s, 1H, H10), 7.52 (d, J = 8 Hz, 1H, H18), 7.37 (d, J = 8 Hz, 1H, H15), 7.33 (d, J = 2 Hz, 1H, H13), 7.21 (t, J = 7 Hz, H16), 7.14 (t, J = 8 Hz, 1H H17), 7.03 (brs, 1H, NH), 7.00 (dd, J = 8, 2 Hz, 1H, H12), 6.93–6.90 (m, 2H, H11, H14), 5.63 (s, 1H, H1), 4.69 (t, J = 7 Hz, 2H, H5), 4.21 (q, J = 7 Hz, 2H, H8), 4.11 (dd, J = 18, 5 Hz, 1H, H7), 4.01 (dd, J = 18, 5 Hz, 1H, H7), 3.72 (s, 2H, H2), 3.65 (t, J = 7 Hz, 2H, H4), 3.36 (t, J = 7 Hz, 2H, H6), 3.03–2.85 (m, 2H, H3), 1.28 ppm (t, J = 7 Hz, 3H, H9). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.5 (C28), 167.7 (C27), 167.6 (C26), 142.8 (C19), 136.3 (C25), 135.2 (C10), 134.9 (C21), 133.7 (C20, C22), 131.8 (C11), 129.6 (C14), 127.4 (C12), 126.9 (C24), 123.1 (C13), 122.6 (C16), 120.0 (C17), 118.2 (C18), 111.6 (C23), 111.5 (C15), 61.8 (C8), 57.6 (C1), 54.2 (C5), 49.6 (C4), 41.9 (C7), 41.1 (C2), 33.6 (C6), 25.2 (C3), 14.3 ppm (C9). HRMS calculated for $C_{28}H_{30}Cl_3N_6O_4$: 619.1394 $[M+H]^+$. Found: 619.1414.

Ugi Adduct 5e

288 mg (0.44 mmol, 76%) of **5e** were obtained from 4-(2-aminoethyl)benzenesulfonamide (115 mg, 0.57 mmol) as a yellow solid. Purification was accomplished through flash chromatography (100:0 to 96:4 CH_2Cl_2/MeOH gradient).^{1}H NMR (400 MHz, CD_3OD) (mixture of rotamers): δ = 7.86–7.74 (m, 4H, H14, H15), 7.35–7.17 (m, 2H, H12, H13), 7.04 (s, 2H, H10, H11), 5.94 (s, 0.65H, H1), 5.92 (s, 0.35H, H1), 4.76 (t, J = 7 Hz, 2H, H5), 4.25 (s, 1H, H2), 4.23 (s, 1H, H2), 4.17 (q, J = 7 Hz, 2H, H8), 4.03–3.88 (m, 2H, H7), 3.66–3.53 (m, 2H, H4), 3.42–3.35 (m, 2H, H6), 2.91–2.77 (m, 1H, H3), 2.63–2.52 (m, 1H, H3), 1.25 ppm (t, J = 7 Hz, 3H, H9). ^{13}C NMR (101 MHz, CD_3OD) (mixture of rotamers): δ = 171.1 (C24), 170.2 (C23), 169.9 (C23), 169.6 (C22), 169.3 (C22), 145.1 (C16), 144.0 (Cq), 143.9 (Cq), 136.4 (Cq), 136.1 (Cq), 135.9 (Cq), 135.3 (Cq), 134.5 (Cq), 133.2 (C10), 130.5 (CAr), 130.3 (CAr), 130.2 (CAr), 128.4 (CAr), 127.5 (CAr), 127.3 (CAr), 62.5 (C8), 62.4 (C8), 58.5 (C1), 57.8 (C1), 55.2 (C5), 55.0 (C5), 50.5 (C4), 42.5 (C2), 42.4 (C7), 36.3 (C3), 34.1 (C6), 14.5 ppm (C9). HRMS calculated for $C_{26}H_{29}Cl_3N_6NaO_6S$: 681.0833 $[M+Na]^+$. Found: 681.0810.

Synthesis of Cyclized Intermediates 4a–e

General Procedure

To a solution of the corresponding Ugi adduct **5** (1.00 mmol) in dry THF (20 mL), DBU (3.00 mmol) was added under an argon atmosphere. After stirring for 15 h at room temperature, the crude reaction mixture was concentrated in vacuo. The resulting residue was taken up in CH_2Cl_2 (15 mL) and washed with 1N HCl (2×10 mL). The organic layer was dried over $MgSO_4$ and filtered. Concentration under reduced pressure afforded crude compounds, which were used without further purification.

1-(3,3-Diphenylpropyl)-2-oxo-4-[N-(2′,4′-dichlorophenetyl)-2H-1,2,3-triazol-4′-yl]-4-(ethoxycarbonylmethylaminocarbonyl)-azetidine (4a)

48 mg (0.08 mmol, 76%) of **4a** were obtained from **5a** (67 mg, 0.10 mmol) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.59 (s, 1H, H11), 7.36 (d, J = 2 Hz, 1H, H14), 7.26–7.22 (m, 4H, HAr), 7.21–7.12 (m, 6H, HAr), 7.05 (dd, J = 6, 2 Hz, 1H, H13), 6.90 (d, J = 7 Hz, 1H, H12), 4.62 (t, J = 6 Hz, 2H, H6), 4.21 (q, J = 6 Hz, 2H, H9), 4.03–3.97 (m, 2H, H8), 3.90 (t, J = 6 Hz, 1H, H3), 3.47 (d, J = 12 Hz, 1H, H2), 3.33 (d, J = 12 Hz, 1H, H2), 3.30 (t, J = 6 Hz, 1H, H7), 3.22–3.14 (m, 1H, H5), 3.11–3.04 (m, 1H, H5), 2.49–2.29 (m, 2H, H4), 1.29 ppm (m, J = 6 Hz, 3H, H10). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.4 (CO), 169.3 (CO), 166.9 (C23), 144.7 (C18), 143.9 (C22), 143.8 (C22), 135.0 (C20), 133.8 (C21), 133.6 (C11), 133.5 (C19), 131.7 (C12), 129.6 (C14), 128.7 (CAr), 128.6 (CAr), 127.9 (CAr), 127.8 (CAr), 127.4 (C13), 126.5 (CAr), 126.4 (CAr), 61.9 (C9), 58.8 (C1), 54.4 (C6), 50.6 (C2), 49.0 (C3), 41.8 (C8), 41.7 (C5), 33.5 (C7), 33.3 (C4), 14.3 ppm (C10). HRMS calculated for $C_{33}H_{33}Cl_2N_5NaO_4$: 656.1807 $[M+Na]^+$. Found: 6561822.

1-(4′-fluorophenethyl)-2-oxo-4-[N-(2′,4′-dichlorophenetyl)-2H-1,2,3-triazol-4′-yl]-4-(ethoxycarbonylmethylaminocarbonyl)-azetidine (4b)

155 mg (0.28 mmol, 91%) of **4b** were obtained from **5b** (181 mg, 0.30 mmol) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.57 (s, 1H, H10), 7.35–7.33 (m, 1H, H13), 7.17–7.12 (m, 2H, H14), 7.08–7.05 (m, 1H, H12), 6.99–6.92 (m, 3H, H11, H15), 6.72 (brs, 1H, NH), 4.68 (t, J = 7 Hz, 2H, H5), 4.21 (q, J = 7 Hz, 2H, H8), 3.95 (dd, J = 18, 6 Hz, 1H, H7), 3.87 (dd, J = 18, 6 Hz, 1H, H7), 3.45–3.29 (m, 5H, H2, H4, H6), 3.24–3.15 (m, 1H, H4), 3.07–2.98 (m, 1H, H3), 2.93–2.84 (m, 1H, H3), 1.29 ppm (t, J = 7 Hz, 3H, H9). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.5 (CO), 169.2 (CO), 167.3 (C22), 161.8 (d, $J_{C\text{-}F}$ = 245 Hz, C21), 144.5 (C16), 135.0 (C18), 134.4 (d, $J_{C\text{-}F}$ = 3 Hz, C20), 133.8 (C19), 133.7 (C10), 133.6 (C17), 131.7 (C11), 130.4 (d, $J_{C\text{-}F}$ = 8 Hz, C14), 129.7 (C13), 127.4 (C12), 115.6 (d, $J_{C\text{-}F}$ = 21 Hz, C15), 61.9 (C8), 59.0 (C1), 54.4 (C5), 50.7 (C2), 45.0 (C4), 41.7 (C7), 33.6 (C6), 33.1 (C3), 14.3 ppm (C9). HRMS calculated for $C_{26}H_{27}Cl_2FN_5O_4$: 562.1424 $[M+H]^+$. Found: 562.1425.

1-(4′-methoxyphenethyl)-2-oxo-4-[N-(2′,4′-dichlorophenetyl)-2H-1,2,3-triazol-4′-yl]-4-(ethoxycarbonylmethylaminocarbonyl)azetidine (4c)

197 mg (0.34 mmol, 83%) of **4c** were obtained from **5c** (250 mg, 0.41 mmol) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.60 (s, 1H, H10), 7.36 (d, J = 2 Hz, 1H, H13), 7.16–7.13 (m, 2H, H14), 7.06 (dd, J = 8, 2 Hz, 1H, H12), 6.93 (d, J = 12 Hz, H11) 6.85–6.80 (m, 2H, H15), 6.45 (t, J = 6 Hz, 1H, NH), 4.68 (t, J = 7 Hz, 2H, H5), 4.20 (q, J = 7 Hz, 2H, H8), 3.85 (dd, J = 18, 6 Hz, 1H, H7), 3.79–3.72 (m, 4H, H7, H16), 3.45–3.30 (m, 5H, H2, H4, H6), 3.13–3.01 (m, 2H, H4, H3), 2.88–2.81 (m, 1H, H3), 1.29 ppm (t, J = 7 Hz, 3H, H9). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.7 (CO), 169.1 (CO), 167.8 (C23), 158.6 (C22), 144.4 (C17), 135.0 (C19), 134.0 (C10), 133.7 (C20), 133.6 (C18), 131.8 (C11), 130.7 (C21), 130.1 (C14), 129.7 (C13), 127.4 (C12), 114.2 (C15), 61.8 (C8), 58.9 (C1), 55.4 (C16), 54.3 (C5), 50.8 (C2), 45.5 (C4), 41.7 (C7), 34.0 (C6), 32.9 (C3), 14.3 ppm (C9). HRMS calculated for $C_{27}H_{30}Cl_2N_5O_5$: 574.1624 $[M+H]^+$. Found: 574.1630.

1-[2′-(1H-indol-3-yl)ethyl]-2-oxo-4-[N-(2′,4′-dichlorophenethyl)-2H-1,2,3-triazol-4′-yl]-4-(ethoxycarbonylmethylaminocarbonyl)azetidine (4d)

165 mg (0.28 mmol, 81%) of **4d** were obtained from **5d** (220 mg, 0.35 mmol) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.17 (brs, 1H, NH), 7.64 (s, 1H, H10), 7.52 (d, J = 8 Hz, 1H, H18), 7.39–7.34 (m, 1H, H13, H15), 7.24 (d, J = 4 Hz, 1H, H14), 7.22–7.17 (m, 1H, H16), 7.14–7.05 (m, 2H, H12, H17), 6.92 (d, J = 8 Hz, 1H, H11), 6.35 (t, J = 6 Hz, 1H, NH), 4.65 (t, J = 7 Hz, 2H, H5), 4.11 (q, J = 7 Hz, 2H, H8), 3.67–3.59 (m, 1H, H4), 3.46 (dd, J = 18, 6 Hz, 1H, H7), 3.40–3.30 (m, 5H, H2, H3, H6), 3.20–3.02 (m, 3H, H3, H4, H7), 1.25 ppm (t, J = 7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.8 (CO), 169.0 (CO), 167.9 (C26), 144.2 (C19), 136.4 (C24), 135.9 (C21), 134.2 (C10), 133.7 (C22), 133.6 (C20), 131.8 (C11), 129.6 (C13), 127.4 (C12), 126.8 (C25), 123.3 (C14), 122.7 (C16), 120.0 (C17), 118.8 (C18), 112.3 (C23), 111.4 (C15), 61.5 (C8), 59.0 (C1), 54.2 (C5), 50.9 (C2), 43.5 (C4), 41.0 (C7), 33.6 (C6), 23.4 (C3), 14.3 ppm (C9). HRMS calculated for $C_{28}H_{29}Cl_2N_6O_4$: 583.1627 $[M+H]^+$. Found: 583.1646.

1-(4′-aminosulfonylphenethyl)-2-oxo-4-[N-(2′,4′-dichlorophenethyl)-2H-1,2,3-triazol-4′-yl]-4-(ethoxycarbonylmethylaminocarbonyl)azetidine (4e)

191 mg (0.31 mmol, 70%) of **4e** were obtained from **5e** (288 mg, 0.44 mmol) as a yellow oil. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.81 (s, 1H, H10), 7.79–7.75 (m, 2H, H15), 7.34 (d, J = 2 Hz, 1H, H13), 7.28–7.24 (m, 2H, H14), 7.13–7.06 (m, 2H, H11, H12), 4.74 (t, J = 7 Hz, 2H, H5), 4.20 (q, J = 7 Hz, 2H, H8), 3.99 (d, J = 18 Hz, 1H, H7), 3.92 (d, J = 18 Hz, 1H, H7), 3.55 (d, J = 15 Hz, 1H, H2), 3.45–3.35 (m, 5H, H2, H4, H6), 2.87 (t, J = 8 Hz, 2H, H3), 1.27 ppm (t, J = 7 Hz, 3H, H9). ^{13}C NMR (101 MHz, CD_3OD): δ = 172.1 (C23), 170.9 (C24), 168.8 (C22), 145.4 (C16), 144.8 (C20), 143.0 (C21), 135.9 (C18), 135.4 (C17), 135.1 (C10), 134.4 (C19), 133.2 (C11), 130.3 (C14), 130.2 (C13), 128.4 (C12), 127.3 (C15), 62.5 (C8), 60.5 (C1), 55.3 (C5), 49.5 (C2), 44.6 (C4), 42.4 (C7), 34.7 (C3), 34.0 (C6), 14.5 ppm (C9). HRMS calculated for $C_{26}H_{28}Cl_2N_6NaO_6S$: 645.1066 $[M+Na]^+$. Found: 645.1086.

Synthesis of Acids 2a–e

General Procedure

To a solution of the corresponding ethyl ester (1 mmol) in 1:1 THF/H_2O (10 mL), LiOH (2.25 mmol) was added. After stirring for 6 h at room temperature, the crude reaction mixture was concentrated in vacuo. The resulting residue was treated with 1 N HCl (10 mL), and the aqueous phase was extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were dried over $MgSO_4$ and filtered. Concentration under reduced pressure afforded the crude compounds, which were used without further purification.

1-(3,3-Diphenylpropyl)-2-oxo-4-[N-(2′,4′-dichlorophenetyl)-2H-1,2,3-triazol-4′-yl]-4-(hydroxycarbonylmethylaminocarbonyl)-azetidine (2a)

160 mg (0.26 mmol, 94%) of **2a** were obtained from **4a** (180 mg, 0.28 mmol) as an orange solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.61 (s, 1H, H9), 7.36 (d, J = 2.1 Hz, 1H, H16), 7.24–7.21 (m, 4H, H12), 7.18–7.14 (m, 6H, H11, H13), 7.09 (t, J = 5.2 Hz, 1H, NH), 7.05 (dd, J = 2.1, 8.2 Hz, 1H, H18), 6.89 (d, J = 8.2 Hz, 1H, H19), 4.60 (t, J = 7.1 Hz, 2H, H6), 4.05 (dd, J = 5.3, 1.6 Hz, 2H, H8), 3.88 (t, J = 7.8 Hz, 1H, H3), 3.46 (d, J = 14.5 Hz, 1H, H2), 3.35 (d, J = 14.5 Hz, 1H, H2), 3.28 (t, J = 7.1 Hz, 2H, H7), 3.14 (m, 1H, H5), 3.09 (m, 1H, H5),

2.35 ppm (m, 2H, H4). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.1 (C23), 169.3 (C21), 167.2 (C22), 144.1 (C20), 143.5 (C10), 134.7 (C15), 133.5 (C17), 133.3 (C14), 131.4 (C19), 129.3 (C16), 127.6 (C18), 127.5 (C11), 128.4 (CAr), 126.3 (CAr), 58.5 (C1), 53.8 (C6), 48.6 (C3), 41.3 (C5), 33.0 (C7), 32.7 ppm (C4). HRMS calculated for $C_{31}H_{29}Cl_2N_5O_4$: 606.1675 $[M+H]^+$. Found: 606.1711.

1-(4′-fluorophenethyl)-2-oxo-4-[N-(2′,4′-dichlorophenetyl)-2H-1,2,3-triazol-4′-yl]-4-(hydroxycarbonylmethylaminocarbonyl)-azetidine (2b)

103 mg (0.19 mmol, 70%) of **2b** were obtained from **4b** (155 mg, 0.28 mmol) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.59 (s, 1H, H8), 7.34 (d, J = 2 Hz, 1H, H11), 7.15–7.10 (m, 2H, H12), 7.07 (dd, J = 8, 2 Hz, 1H, H10), 6.99–6.92 (m, 3H, H9, H13), 6.84–6.79 (m, 1H, NH), 4.68 (t, J = 7 Hz, 2H, H5), 4.07–3.89 (m, 2H, H7), 3.47-3.31 (m, 5H, H2, H4, H6), 3.26–3.16 (m, 1H, H4), 3.04–2.94 (m, 1H, H3), 2.91–2.82 ppm (m, 1H, H3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 172.2 (C22), 169.9 (C21), 167.9 (C20), 161.8 (d, $J_{F\text{-}C}$ = 245 Hz, C19), 144.1 (C14), 135.0 (C15), 134.3 (C17), 134.2 (C18), 133.9 (C8), 133.5 (C16), 131.7 (C9), 130.4 (d, $J_{F\text{-}C}$ = 8 Hz, C12), 129.7 (C11), 127.4 (C10), 115.6 (d, $J_{F\text{-}C}$ = 21 Hz, C13), 59.1 (C1), 54.4 (C5), 50.5 (C2), 45.0 (C4), 41.5 (C7), 33.6 (C6), 33.0 ppm (C3). HRMS calculated for $C_{24}H_{22}Cl_2FN_5NaO_4$: 556.0931 $[M+Na]^+$. Found: 556.0935.

1-(4′-methoxyphenethyl)-2-oxo-4-[N-(2′,4′-dichlorophenetyl)-2H-1,2,3-triazol-4′-yl]-4-(hydroxycarbonylmethylaminocarbonyl)azetidine (2c)

110 mg (0.20 mmol, 69%) of **2c** were obtained from **4c** (197 mg, 0.34 mmol) as a yellow waxy solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.61 (s, 1H, H8), 7.36–7.35 (m, 1H, H11), 7.14–7.10 (m, 2H, H12), 7.07 (dd, J = 8, 2 Hz, 1H, H10), 6.93 (d, J = 8 Hz, 1H, H9), 6.85–6.80 (m, 2H, H13), 6.61–6.53 (m, 1H, NH), 4.67 (t, J = 7 Hz, 2H, H15), 3.94–3.73 (m, 5H, H7, H14), 3.45–3.31 (m, 5H, H2, H4, H6), 3.13–2.99 (m, 2H, H4, H3), 2.87–2.76 ppm (m, 1H, H3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 172.2 (C23), 170.1 (C22), 168.3 (C21), 158.6 (C20), 144.1 (C15), 135.0 (C17), 134.0 (C8), 133.7 (C18), 133.6 (C16), 131.8 (C9), 130.5 (C18), 130.1 (C12), 129.6 (C11), 127.4 (C10), 114.3 (C13), 59.1 (C1), 55.4 (C14), 54.3 (C5), 50.6 (C2), 45.5 (C4), 41.5 (C7), 33.6 (C6), 32.8 ppm (C3). HRMS calculated for $C_{25}H_{25}Cl_2N_5O_5$: 546.1311 $[M+H]^+$. Found: 546.1332.

1-[2′-(1H-indol-3-yl)ethyl]-2-oxo-4-[N-(2′,4′-dichlorophenethyl)-2H-1,2,3-triazol-4′-yl]-4-(hydroxycarbonylmethylaminocarbonyl)azetidine (2d)

125 mg (0.23 mmol, 81%) of **2d** were obtained from **4d** (165 mg, 0.28 mmol) as a yellow solid. In this case, purification was afforded by flash chromatography (100:0 to 96:4 CH_2Cl_2/MeOH gradient). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.74 (s, 1H, H8), 7.45 (dt, J = 8, 4 Hz, 1H, H16), 7.34–7.30 (m, 2H, H11, H13), 7.10–7.05 (m, 1H, H15), 7.03-6.95 (m, 4H, H9, H10, H12, H14), 4.62 (t, J = 7 Hz, 2H, H5), 3.79 (dt, J = 18, 3 Hz, 1H, H7), 3.65 (dt, J = 18, 4 Hz, 1H, H7), 3.53–3.36 (m, 4H, H2, H4), 3.29 (t, J = 7 Hz, 2H, H6), 3.10–3.01 (m, 1H, H3), 2.94–2.85 ppm (m, 1H, H3). ^{13}C NMR (101 MHz, CD_3OD): δ = 172.4 (C26), 172.2 (C25), 169.3 (C24), 145.5 (C17), 138.1 (C_q), 135.9 (C_q), 135.4 (C_q), 135.1 (C8), 134.5 (C_q), 133.2 (C9), 130.2 (C11), 128.4 (C10), 128.3 (C_q), 123.8 (C12), 122.5 (C15), 119.8 (C14), 119.3 (C16), 112.5 (C_q), 112.3 (C13), 60.4 (C1), 55.1 (C5), 49.8 (C2), 44.7 (C4), 42.0 (C7), 34.1 (C6), 24.7 ppm (C3). HRMS calculated for $C_{26}H_{24}Cl_2N_6NaO_4$: 577.1134 $[M+Na]^+$. Found: 577.1141.

1-(4′-aminosulfonylphenethyl)-2-oxo-4-[N-(2′,4′-dichlorophenethyl)-2H-1,2,3-triazol-4′-yl]-4-(hydroxycarbonylmethylaminocarbonyl)azetidine (2e)

120 mg (0.20 mmol, 66%) of **2e** were obtained from **4e** (191 mg, 0.31 mmol) as a white solid. In this case, the reaction mixture was concentrated *in vacuo* and purified by reversed-phase chromatography (95:5 to 40:60 H_2O/ACN gradient). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.81 (s, 1H, H8), 7.79–7.75 (m, 2H, H13), 7.33 (d, J = 2 Hz, 1H, H11), 7.30–7.25 (m, 2H, H12), 7.13–7.05 (m, 2H, H9, H10), 4.74 (t, J = 7 Hz, 2H, H5), 3.79 (d, J = 17 Hz, 1H, H7), 3.72 (d, J = 17 Hz, 1H, H7), 3.51–3.35 (m, 6H, H2, H4, H6), 2.92–2.85 ppm (m, 2H, H3). ^{13}C NMR (101 MHz, CD_3OD): δ = 175.6 (C22), 170.7 (C21), 169.3 (C20), 145.8 (C14), 144.9 (C18), 143.1 (C19), 135.9 (C16), 135.5 (C17), 135.1 (C8), 134.5 (C15), 133.3 (C9), 130.4 (C12), 130.2 (C11), 128.4 (C10), 127.3 (C13), 60.6 (C1), 55.3 (C5), 49.5 (C2), 44.9 (C7), 44.7 (C4), 34.7 (C3), 34.1 ppm (C6). HRMS calculated for $C_{24}H_{24}Cl_2N_6NaO_6S$: 617.0753 $[M+H]^+$. Found: 617.0794.

Synthesis of Acylated Derivatives 3aA–3eA and 3aB–3eB

General Procedure

To a solution of the corresponding carboxylic acid **2** (0.12 mmol) and HOBt (0.14 mmol) in dry CH_2Cl_2 (5 mL), DIPEA (0.29 mmol) was added. Next, EDC (0.14 mmol) was added, followed by methoxyethanamine (**3a–eA**) or *N*-(2-*a*minoethyl)acetamide (**3a–eB**) (0.12 mmol). The reaction mixture was stirred for 15 h at room temperature under an argon atmosphere. The mixture was next diluted by addition of CH_2Cl_2 (10 mL), and washed successively with H_2O (10 mL) and brine (10 mL), unless otherwise indicated. The organic layer was dried over $MgSO_4$ and filtered. Concentration under reduced pressure afforded crude compounds, which were purified as indicated.

1-(3′,3′-Diphenylpropyl)-2-oxo-4-[N-(2′,4′-dichlorophenetyl)-2H-1,2,3-triazol-4′-yl]-4-((2′-methoxyethyl)aminocarbonylmethylamino carbonyl)azetidine (3aA)

50 mg (0.08 mmol, 63%) of **3aA** were obtained from **2a** (71 mg, 0.12 mmol) as a white waxy solid. The compound was purified by flash chromatography (100:0 to 95:5 CH_2Cl_2/MeOH gradient). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.57 (s, 1H, H12), 7.36 (d, J = 2 Hz, 1H, H15), 7.30–7.11 (m, 11H, HAr), 7.06 (dd, J = 8, 2 Hz, 1H, H14), 6.91 (d, J = 8 Hz, 1H, H13), 6.28-6.18 (m, 1H, NH), 4.62 (t, J = 7 Hz, 2H, H6), 3.93–3.85 (m, 3H, H3, H8), 3.48–3.42 (m, 5H, H2, H9, H10), 3.35–3.27 (m, 6H, H2, H7, H11), 3.20–3.06 (m, 2H, H5), 2.47–2.27 ppm (m, 2H, H4). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.5 (C26), 167.9 (C25), 166.7 (C24), 144.8 (C19), 144.0 (C23), 143.9 (C23), 135.0 (C21), 133.8 (C22), 133.6 (C20), 133.4 (C12), 131.8 (C13), 129.6 (C15), 128.7 (CAr), 128.6 (CAr), 127.9 (CAr), 127.8 (CAr), 127.4 (C14), 126.5 (C18), 70.9 (C10), 58.9 (C11), 58.8 (C1), 54.4 (C6), 50.4 (C2), 49.0 (C3), 43.3 (C8), 41.7 (C5), 39.5 (C9), 33.5 (C7), 33.4 ppm (C4). HRMS calculated for $C_{34}H_{36}Cl_2N_6NaO_4$: 685.2073 $[M+Na]^+$. Found: 685.2055.

1-(3′,3′-Diphenylpropyl)-2-oxo-4-[N-(2′,4′-dichlorophenetyl)-2H-1,2,3-triazol-4′-yl]-4-((2′-acetamidylethyl)aminocarbonyl-methylamino carbonyl)azetidine (3aB)

31 mg (0.05 mmol, 42%) of **3aB** were obtained from **2a** (64 mg, 0.11 mmol) as a white waxy solid. The compound was purified by flash chromatography (100:0 to 95:5 CH_2Cl_2/MeOH gradient). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.60 (s, 1H, H12), 7.35 (d, J = 2 Hz,

1H, H15), 7.34–7.31 (m, 1H, NH), 7.26–7.21 (m, 4H H17), 7.20–7.11 (m, 6H, H16, H18), 7.06 (dd, $J=8$, 2 Hz, 1H, H14), 6.91 (d, $J=8$ Hz, 1H, H13), 6.51 (brs, 1H, NH), 4.60 (t, $J=7$ Hz, 2H, H6), 3.92–3.85 (m, 3H, H3, H8), 3.48 (d, $J=14$ Hz, 1H, H2), 3.38–3.26 (m, 7H, H2, H7, H9, H10), 3.20–3.07 (m, 2H, H5), 2.37–2.27 (m, 2H, H4), 1.93 ppm (s, 3H, H11). ^{13}C NMR (101 MHz, $CDCl_3$): $\delta=172.6$ (C27), 169.8 (CO), 169.0 (CO), 166.9 (C24), 144.8 (C19), 144.0 (C23), 143.9 (C23), 135.0 (C21), 133.8 (C12), 133.6 (C20), 133.5 (C22), 131.8 (C13), 129.6 (C15), 128.8 (CAr), 128.7 (CAr), 128.6 (CAr), 127.9 (CAr), 127.8 (CAr), 127.7 (C14), 126.6 (CAr), 126.5 (CAr), 59.0 (C1), 54.4 (C6), 50.3 (C2), 49.0 (C3), 43.5 (C8), 41.7 (C5), 40.2 (C9), 39.9 (C10), 33.5 (C7), 33.4 (C4), 22.8 ppm (C11). HRMS calculated for $C_{35}H_{37}Cl_2N_7NaO_4$: 712.2182 $[M+Na]^+$. Found: 712.2178.

*1-(4′-fluorophenethyl)-2-oxo-4-[*N*-(2′,4′-dichlorophenetyl)-2*H*-1,2,3-triazol-4′-yl]-4-((2′-methoxyethyl)aminocarbonylmethylamino carbonyl)azetidine (3 bA)*

24 mg (0.04 mmol, 53%) of **3 bA** were obtained from **2 b** (41 mg, 0.08 mmol) as a yellow oil. The compound was purified by flash chromatography (100:0 to 97:3 CH_2Cl_2/MeOH gradient). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.53$ (s, 1H, H11), 7.34 (d, $J=2$ Hz, 1H, H14), 7.14–7.09 (m, 2H, H15), 7.07 (dd, $J=8$, 2 Hz, 1H, H13), 7.04–7.00 (m, 1H, NH), 6.98–6.92 (m, 3H, H12, H16), 6.23–6.15 (m, 1H, NH), 4.68 (t, $J=7$ Hz, 2H, H5), 3.88 (d, $J=5$ Hz, 2H, H7), 3.49–3.43 (m, 4H, H8, H9), 3.42–3.27 (m, 9H, H2, H4, H6, H10), 3.01–2.84 ppm (m, 2H, H3). ^{13}C NMR (101 MHz, $CDCl_3$): $\delta=169.5$ (C25), 167.8 (C24), 167.0 (C23), 161.8 (d, $J_{F\text{-}C}=246$ Hz, C22), 144.6 (C17), 135.0 (C19), 134.4 (d, $J_{F\text{-}C}=3$ Hz, C21), 133.8 (C18), 133.6 (C20), 133.5 (C11), 131.7 (C12), 130.4 (C15), 130.3 (C15), 129.7 (C14), 127.4 (C13), 115.6 (C16), 115.4 (C16), 70.9 (C9), 59.0 (C1), 58.9 (C10), 54.4 (C5), 50.4 (C2), 44.8 (C4), 43.3 (C7), 39.5 (C8), 33.5 (C6), 33.2 ppm (C3). HRMS calculated for $C_{27}H_{29}Cl_2FN_6NaO_4$: 613.1509 $[M+Na]^+$. Found: 613.1520.

*1-(4′-fluorophenethyl)-2-oxo-4-[*N*-(2′,4′-dichlorophenetyl)-2*H*-1,2,3-triazol-4′-yl]-4-((2′-acetamidylethyl)aminocarbonylmethylamino carbonyl)azetidine (3 bB)*

27 mg (0.06 mmol, 53%) of **3 bB** were obtained from **2 b** (44 mg, 0.08 mmol) as colorless oil. The compound was purified by flash chromatography (100:0 to 92:8 CH_2Cl_2/MeOH gradient). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.56$ (s, 1H, H11), 7.34 (d, $J=2$ Hz, 1H, H14), 7.14–7.10 (m, 2H, H15), 7.07 (dd, $J=8$, 2 Hz, 1H, H13), 7.03–6.99 (m, 1H, NH), 6.97–6.92 (m, 3H, H13, H16), 6.35 (brs, 1H, NH), 4.68 (t, $J=7$ Hz, 2H, H5), 3.85 (d, $J=5$ Hz, 2H, H7), 3.51–3.25 (m, 10H, H2, H4, H6, H8, H9), 3.02–2.83 ppm (m, 2H, H3), 1.95 (s, 3H, H10). ^{13}C NMR (101 MHz, $CDCl_3$): $\delta=172.8$ (C26), 169.9 (CO), 169.1 (CO), 167.3 (C23), 161.7 (d, $J_{F\text{-}C}=246$ Hz, C22), 144.6 (C17), 134.9 (CAr), 134.3 (d, $J_{F\text{-}C}=3$ Hz, C21), 133.8 (CAr), 133.6 (C11), 133.5 (CAr), 131.8 (C12), 130.4 (C15), 130.3 (C15), 129.6 (C14), 127.4 (C13), 115.6 (C16), 115.4 (C16), 59.1 (C1), 54.4 (C5), 50.2 (C2), 44.7 (C4), 43.4 (C7), 40.2 (C8), 40.0 (C9), 33.5 (C6), 33.2 (C3), 22.7 ppm (C10). HRMS calculated for $C_{28}H_{31}Cl_2FN_7O_4$: 618.1799 $[M+H]^+$. Found: 618.1795.

*1-(4′-methoxyphenethyl)-2-oxo-4-[*N*-(2′,4′-dichlorophenetyl)-2*H*-1,2,3-triazol-4′-yl]-4-((2′-methoxyethyl)aminocarbonylmethylamino carbonyl)azetidine (3 cA)*

40 mg (0.07 mmol, 57%) of **3 cA** were obtained from **2 c** (63 mg, 0.12 mmol), as a yellow oil. The compound was purified by flash chromatography (100:0 to 97:3 CH_2Cl_2/MeOH gradient). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.56$ (s, 1H, H11), 7.36 (d, $J=2$ Hz, 1H, H14), 7.13–7.09 (m, 2H, H15), 7.07 (dd, $J=8$, 2 Hz, 1H, H13), 6.95 (d, $J=8$ Hz, 1H, H12), 6.85–6.76 (m, 3H, H16), 6.16 (brs, 1H, NH), 4.68 (t, $J=7$ Hz, 2H, H5), 3.80–3.76 (m, 5H, H7, H17), 3.46–3.33 (m, 11H, H2, H4, H8, H9, H10), 3.19–3.10 (m, 1H, H4), 3.06–2.97 (m, 1H, H3), 2.88–2.80 ppm (m, 1H, H3). ^{13}C NMR (101 MHz, $CDCl_3$): $\delta=169.7$ (CO), 167.8 (CO), 167.3 (C24), 158.5 (C23), 144.5 (C18), 134.9 (C20), 133.7 (C19), 133.6 (C11), 133.5 (C21), 131.7 (C12), 130.6 (C22), 129.9 (C15), 129.6 (C14), 127.4 (C13), 114.2 (C16), 70.9 (C9), 59.0 (C1), 58.9 (C10), 55.4 (C17), 54.3 (C5), 50.5 (C2), 45.3 (C4), 43.4 (C7), 39.4 (C8), 33.5 (C6), 33.0 ppm (C3). HRMS calculated for $C_{28}H_{33}Cl_2N_6O_5$: 603.1889 $[M+H]^+$. Found: 603.1906.

*1-(4′-methoxyphenethyl)-2-oxo-4-[*N*-(2′,4′-dichlorophenetyl)-2*H*-1,2,3-triazol-4′-yl]-4-((2′-acetamidylethyl)aminocarbonylmethylamino carbonyl)azetidine (3 cB)*

37 mg (0.06 mmol, 53%) of **3 cB** were obtained from **2 c** (59 mg, 0.11 mmol) as a white waxy solid. The compound was purified by flash chromatography (100:0 to 92:8 CH_2Cl_2/MeOH gradient). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.59$ (s, 1H, H11), 7.36–7.33 (m, 1H, H14), 7.12–7.05 (m, 3H, H13, H15), 6.95 (d, $J=8$ Hz, 1H, H12), 6.92–6.87 (m, 1H, NH), 6.83–6.79 (m, 2H, H16), 6.55–6.49 (m, 1H, NH), 4.67 (t, $J=7$ Hz, 2H, H5), 3.79–3.73 (m, 5H, H7, H17), 3.48–3.31 (m, 9H, H2, H4, H6, H8, H9), 3.25–3.15 (m, 1H, H4), 3.05–2.96 (m, 1H, H3), 2.87–2.76 ppm (m, 1H, H3), 1.94 (s, 3H, H10). ^{13}C NMR (101 MHz, $CDCl_3$): $\delta=172.0$ (C27), 170.1 (C25), 168.9 (C26), 167.5 (C2), 158.5 (C23), 144.5 (C18), 134.9 (C20), 133.8 (C11), 133.7 (C19), 133.6 (C21), 131.8 (C12), 130.6 (C22), 129.9 (C15), 129.6 (C14), 127.4 (C13), 114.2 (C16), 59.0 (C1), 55.4 (C17), 54.3 (C5), 50.5 (C2), 45.3 (C4), 43.5 (C7), 40.6 (C8), 39.7 (C9), 33.5 (C6), 33.0 (C3), 23.1 ppm (C10). HRMS calculated for $C_{29}H_{33}Cl_2N_7NaO_5$: 652.1818 $[M+Na]^+$. Found: 652.1822.

*1-[2′-1*H*-indol-3-yl)ethyl]-2-oxo-4-[*N*-(2′,4′-dichlorophenetyl)-2*H*-1,2,3-triazol-4′-yl]-4-((2′-methoxyethyl)aminocarbonylmethylamino carbonyl)azetidine (3 dA)*

17 mg (0.03 mmol, 30%) of compound **3 dA** were obtained from **2 d** (50 mg, 0.09 mmol) as a yellow waxy solid. The compound was purified by flash chromatography (100:0 to 97:3 CH_2Cl_2/MeOH gradient). ^{1}H NMR (400 MHz, CD_3OD): $\delta=7.88$ (t, $J=6$ Hz, 1H, NH), 7.75 (s, 1H, H11), 7.46 (dt, $J=8$, 1 Hz, 1H, H19), 7.35–7.32 (m, 2H, H14, H16), 7.12–7.07 (m, 1H, H18), 7.05–6.97 (m, 4H, H12, H13, H15, H17), 4.66 (t, $J=7$ Hz, 2H, H5), 3.78 (d, $J=16$ Hz, 1H, 2H), 3.64 (d, $J=17$ Hz, 1H, 2H), 3.54–3.43 (m, 6H, H4, H7, H9), 3.41–3.36 (m, 2H, H8), 3.33–3.29 (m, 5H, H6, H10), 3.10–3.02 (m, 1H, H3), 2.97–2.88 ppm (m, 1H, H3). ^{13}C NMR (101 MHz, CD_3OD): $\delta=172.1$ (C27), 170.8 (C29), 169.3 (C26), 145.8 (C20), 138.1 (C25), 135.9 (C21), 135.4 (C22), 134.9 (C11), 134.5 (C23), 133.2 (C14), 130.2 (C12), 128.4 (C24), 123.4 (C15), 122.5 (C18), 119.8 (C17), 119.2 (C19), 112.6 (C26), 112.4 (C16), 72.0 (C9), 60.5 (C1), 58.9 (C10), 55.1 (C5), 49.8 (C7), 44.8 (C4), 43.5 (C2), 40.3 (C8), 34.1 (C6), 24.7 ppm (C3). HRMS calculated for $C_{29}H_{31}Cl_2N_7NaO_4$: 634.1712 $[M+Na]^+$. Found: 634.1724.

*1-[2′-1*H*-indol-3-yl)ethyl]-2-oxo-4-[*N*-(2′,4′-dichlorophenetyl)-2*H*-1,2,3-triazol-4′-yl]-4-((2′-acetamidylethyl)aminocarbonylmethylamino carbonyl)azetidine (3 dB)*

14 mg (0.02 mmol, 24%) of **3 dB** were obtained from **2 d** (50 mg, 0.09 mmol) as a white solid. The compound was purified by flash chromatography (100:0 to 95:5 CH_2Cl_2/MeOH gradient). ^{1}H NMR (400 MHz, CD_3OD): $\delta=8.01$–7.92 (m, 1H, NH), 7.76 (s, 1H, H11), 7.46–7.43 (m, 1H, H19), 7.33–7.30 (m, 2H, H14, H16), 7.10–7.05 (m,

1H, H18), 7.03–6.95 (m, 4H, H12, H13, H15, H17), 4.62 (t, $J=7$ Hz, 2H, H5), 3.72 (d, $J=16$ Hz, 1H, H2), 3.63 (d, $J=16$ Hz, 1H, H2), 3.50–3.42 (m, 4H, H4, H7), 3.30–3.25 (m, 6H, H6, H8, H9), 3.09–3.00 (m, 1H, H3), 2.95–2.86 (m, 1H, H3), 1.90 ppm (s, 3H, H10). ^{13}C NMR (101 MHz, CD_3OD): $\delta=173.7$ (C30), 172.3 (C27), 171.1 (C29), 169.3 (C28), 145.7 (C20), 138.1 (C25), 135.9 (C21), 135.4 (C22), 135.0 (C11), 134.5 (C23), 133.2 (C13), 130.2 (C14), 128.4 (C12), 128.3 (C24), 123.8 (C15), 122.5 (C18), 119.7 (C17), 119.2 (C19), 112.6 (C26), 112.4 (C16), 60.5 (C1), 55.2 (C5), 49.8 (C7), 44.8 (C4), 43.6 (C2), 40.3 (C8), 39.9 (C9), 34.1 (C6), 24.7 (C3), 22.6 ppm (C10). HRMS calculated for $C_{30}H_{32}Cl_2N_8NaO_4$: 661.1821 $[M+Na]^+$. Found: 661.1819.

1-(4′-aminosulfonylphenethyl)-2-oxo-4-[N-(2′,4′-dichlorophenethyl)-2H-1,2,3-triazol-4′-yl]-4-((2′-methoxyethyl)aminocarbonylmethyl aminocarbonyl)azetidine (3eA)

16 mg (0.02 mmol, 31%) of **3eA** were obtained from **2e** (47 mg, 0.08 mmol) in dry DMF, as a white solid. In this case, the reaction mixture was concentrated in vacuo and the residue purified by preparative thin layer chromatography (87:13 CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, CD_3OD): $\delta=7.81$ (s, 1H, H11), 7.79–7.75 (m, 2H, H16), 7.34 (d, $J=1$ Hz, 1H, H14), 7.29–7.25 (m, 2H, H15), 7.13–7.06 (m, 2H, H12, H13), 4.77 (t, $J=7$ Hz, 2H, H5), 3.93–3.81 (m, 2H, H7), 3.49–3.34 (m, 10H, H2, H4, H6, H8, H9), 3.32 (s, 3H, H10), 2.90–2.84 ppm (m, 2H, H3). ^{13}C NMR (101 MHz, CD_3OD): $\delta=171.9$ (C24), 171.0 (C25), 168.9 (C23), 145.7 (C17), 144.9 (C21), 143.1 (C22), 135.9 (C19), 135.4 (C18), 135.0 (C11), 134.5 (C20), 133.3 (C12), 130.3 (C15), 130.2 (C14), 128.4 (C13), 127.3 (C16), 72.0 (C9), 60.6 (C1), 58.9 (C10), 55.3 (C5), 49.5 (C2), 44.7 (C4), 43.6 (C7), 40.3 (C8), 34.7 (C3), 34.1 ppm (C6). HRMS calculated for $C_{27}H_{32}Cl_2N_7O_6S$: 652.1512 $[M+H]^+$. Found: 652.1553.

1-(4′-aminosulfonylphenethyl)-2-oxo-4-[N-(2′,4′-dichlorophenethyl)-2H-1,2,3-triazol-4′-yl]-4-((2′-acetamidylethyl)aminocarbonylmethyl aminocarbonyl)azetidine (3eB)

14 mg (0.02 mmol, 23%) of compound **3eB** were obtained from **2e** (56 mg, 0.09 mmol) in dry DMF, as a white solid. In this case, the reaction mixture was concentrated in vacuo and the residue purified by preparative thin layer chromatography (85:15 CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, CD_3OD): $\delta=7.81$ (s, 1H, H11), 7.79–7.75 (m, 2H, H16), 7.34 (d, $J=2$ Hz, 1H, H14), 7.29–7.24 (m, 2H, H15), 7.13–7.06 (m, 2H, H12, H13), 4.74 (t, $J=7$ Hz, 2H, H5), 3.84 (s, 2H, H7), 3.48 (s, 2H, H2), 3.44 (t, $J=8$ Hz, 2H, H4), 3.38 (t, $J=7$ Hz, 2H, H6), 3.30–3.25 (m, 4H, H8, H9), 2.87 (t, $J=7$ Hz, 2H, H3), 1.92 ppm (s, 3H, H10). ^{13}C NMR (101 MHz, CD_3OD): $\delta=173.7$ (C26), 172.1 (C24), 171.2 (C25), 169.0 (C23), 145.7 (C17), 144.9 (C21), 143.1(C22), 135.9 (C18), 135.4 (C19), 135.0 (C11), 134.5 (C20), 133.3 (C12), 130.3 (C15), 130.2 (C14), 128.4 (C13), 127.3 (C16), 60.6 (C1), 55.3 (C5), 49.5 (C2), 44.7 (C4), 43.7 (C7), 40.3 (C8), 40.0 (C9), 34.8 (C3), 34.1 (C6), 22.6 ppm (C10). HRMS calculated for $C_{28}H_{32}Cl_2N_8NaO_6S$: 701.1440 $[M+Na]^+$. Found: 701.1487.

Apoptosome Reconstitution Assay in Cell Extracts

The apoptosome reconstitution assay in cellular extracts was carried out by using cytosolic extracts (S100) of HEK-293 cells depleted of Apaf-1. Cytosolic extracts from 2×10^8 cells were fractionated by ionic exchanged chromatography in a MonoQ FPLC column (Amershan Pharmacia Biotech). The flow-through (FT) fraction contained caspase-3, caspase-9, and cytochrome *c*; thus, addition of recombinant Apaf-1 and dATP makes the apoptosome reconstitution possible. Test compounds, or the vehicle (1.7 μL in DMSO), were added to 96-well Optiplate969. Next, caspase assay buffer (PBS, 10% glycerol, 0.1 mM EDTA, 2 mM DTT) (90 μL) was added, followed by rApaf-1 (5 ng). After incubation at 30 °C for 30 min, HEK-293 extracts (20 μg) were added, followed by 10 mM dATP. After incubation for 30 min at 37 °C, 2 mM caspase-3 fluorogenic substrate (Ac-DEVD-afc) was added, and caspase-3 activity was continuously measured by the release of afc in a Wallac 1420 Worksation (λ_{exc} 390 nm; λ_{em} 510 nm). The assay was performed in duplicate, and data values were reported as mean ± SD.

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Keywords: apoptosis · caspase-3 inhibitors · intramolecular cyclization · Ugi reaction · β-lactams

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