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Targeted Polypharmacology: Discovery of a Highly Potent Non-Hydroxamate Dual Matrix Metalloproteinase (MMP)-10/-13 Inhibitor

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ABSTRACT

Matrix metalloproteinases (MMPs) play a key role in many diseases like cancer, atherosclerosis or arthritis. Interest in MMP inhibition has been revitalized very recently as the knowledge on the underlying network of biological pathways is steadily growing. Based on this new insight into the relevance of MMP-10 and MMP-13 within the MMP network and the ban of hydroxamate inhibitors from clinical development, the discovery of non-hydroxamate multi-target drugs against specific MMPs is of foremost interest. Here, we disclose the discovery of a

very potent and selective non-hydroxamate MMP-10/-13 inhibitor. The high potency (IC_{50} of 31 nM [MMP-10] and 5 nM [MMP-13]) and selectivity over MMP-1, -2, -3, -7, -8, -9, -12 and -14 enable this compound to decipher disease causing MMP networks and to generate new treatment options through targeted polypharmacology.

INTRODUCTION

The relevance of matrix metalloproteinase (MMP) inhibition for the treatment of diseases has been revitalized recently, mostly validated by knockout experiments showing the importance of specifically interconnected MMPs.[1] The necessity for MMP inhibitors with a specific profile for either one or several MMPs has clearly emerged over time.[1] Unfortunately, potent hydroxamate MMP inhibitors delivered negative outcomes in clinical trials.[1–3] Unspecific inhibition of metal containing proteins by the hydroxamic acid led to severe side effects and finally to the exclusion of these inhibitors form clinical development.[2,3] Recently gained knowledge about the connected functionality within the MMP family asks for inhibitors with defined inhibition profiles.[1,4] The benefits of polypharmacological drugs are well accepted as they show more predictable pharmacokinetics and a lower probability of drug interactions than mixtures of inhibitors.[5,6] This is especially important in complex diseases like neurodegenerative diseases, chronic inflammation or cancer for which disturbed biological networks are the root cause.[7]

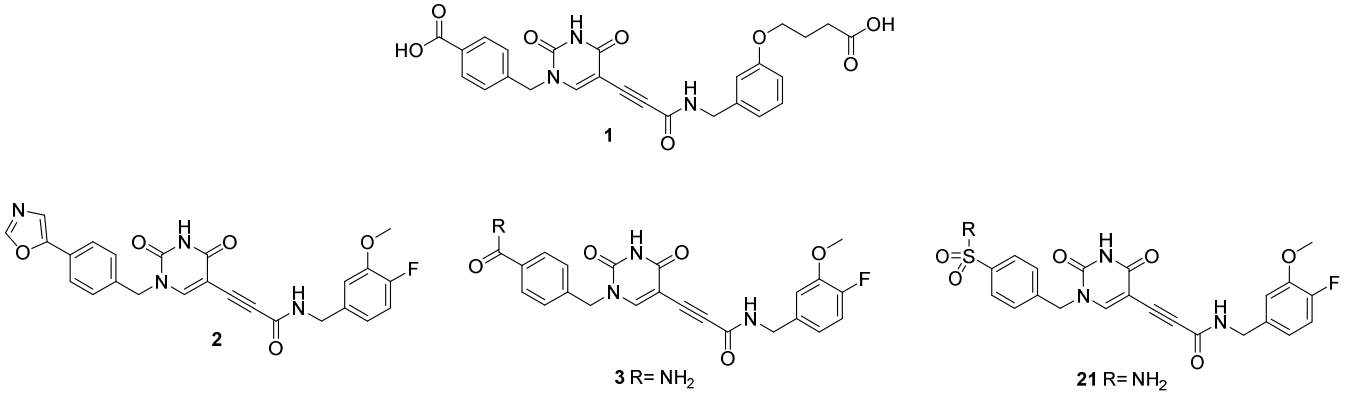
MMPs are major enzymes in the degradation and remodeling of the extracellular matrix. 23 members of the human MMP family are known.[4,8,9] MMPs are zinc containing proteases that possess, next to the catalytically active zinc ion, a S1' selectivity loop, which is hydrophobic in

nature and of different depth among the different MMPs.[8,9] In healthy organisms, MMPs are involved in bone modeling and remodeling, mammary development or blood vessel remodeling, for example. Dysfunction of MMP expression leads to a broad field of devastating diseases like cancer, arthritis or fibrosis.[8,9]

The targeted proteins MMP-10 and -13, also known as stromelysin-2 and collagenase-3, play important roles in those diseases. Both proteins are involved in several types of cancer (lung cancer,[10–14] head and neck cancer,[11,15–17] breast cancer,[16,17] gastric cancer,[16,17] skin cancer,[16,17] esophageal carcinoma[18] or thymic lymphoma[19]), and in arthritis.[16,20,21] In addition, MMP-10 is able to activate the pro-collagenases.[20,22,23] MMP-13 is thereby super activated by MMP-10 and shows about a tenfold higher activity than activated by trypsin or plasmin.[20]

Based on the documented disease relevance of MMP-10 as well as MMP-13, we set out to develop a dual MMP-10/-13 inhibitor for a targeted polypharmacology approach. For MMP-10, no selective and potent inhibitor is described in literature so far, whereas for MMP-13, there are several selective inhibitors known with nanomolar affinity or even better.[24–28] Therefore, we started with a rather poor affinity for MMP-10 based on our previous work[29] (**1**: 6 nM [MMP-13] and 8.40 µM [MMP-10], Figure 1) and employed structure-based design to improve the affinity for MMP-10 and MMP-13 in parallel by reaching into the S1'* pocket of the target proteins to design a dual inhibitor for both proteins.[30] The newly generated carboxamide and sulfonamide derivatives as well as the starting molecule are depicted in figure 1.

Figure 1. Starting molecule **1** from our previous work[29] and newly designed/synthesized structures **2** (oxazole), **3-20** (carboxamides) and **21-28** (sulfonamides).



1

2

3 R= NH_2
4 R= NHMe
5 R= NMe_2
6 R= OMe
7 R= $NHCH(CH_3)_2$
8 R= NHcycProp
9 R= $NHC(CH_3)_3$
10 R= $NH(CH_2)_2OH$
11 R= Gly-OMe
12 R= L-Ala-OMe
13 R= L-Ser-OMe
14 R= D-Pro-OMe
15 R= Gly-OH
16 R= L-Ala-OH
17 R= D-Ala-OH
18 R= L-Ser-OH
19 R= D-Ser-OH
20 R= D-Pro-OH

21 R= NH_2
22 R= NHMe
23 R= NMe_2
24 R= $NHCH(CH_3)_2$
25 R= NHcycProp
26 R= $NHC(CH_3)_3$
27 R= morpholine
28 R= Gly-OMe

RESULTS AND DISCUSSION

Molecular modeling. Docking studies were performed with the combination of Chimera[31] and AutoDock Vina.[32] The docking parameters as well as the design setup can be found in the supporting information. The MMP-10 structure with PDB code 1Q3A[33] and the MMP-13 structure with PDB code 456C[34] were used.

The design of the synthesized compounds **2-28** was based on compound **1**, from which the zinc chelating acid functionality on the right hand site was removed and the phenyl ring was substituted by fluorine in para position to enable more stability against degradation. The main

changes for the optimization in this polypharmacological approach were introduced at the carboxylic acid moiety on the left hand site of **1**. In a first iteration, this carboxylic acid was converted into the primary carboxamide **3**, which indicated the desired positioning into the S1'* pocket of MMP-10 (Figure 2 (a)). The carboxamide was chosen because of the lower polarity compared to the carboxylic acid fragment and the possibility for further H-bond interactions. In the next step, the carboxamide was docked in methylated forms to investigate the accessible space for enlargements into the S1'* pocket. The docking pose shown in figure 2 (b) illustrates the corresponding binding orientation of the dimethylated carboxamide **5**. Based on these docking studies, the extended carboxamides **3-20** as well as the related sulfonamides **21-28** were designed.

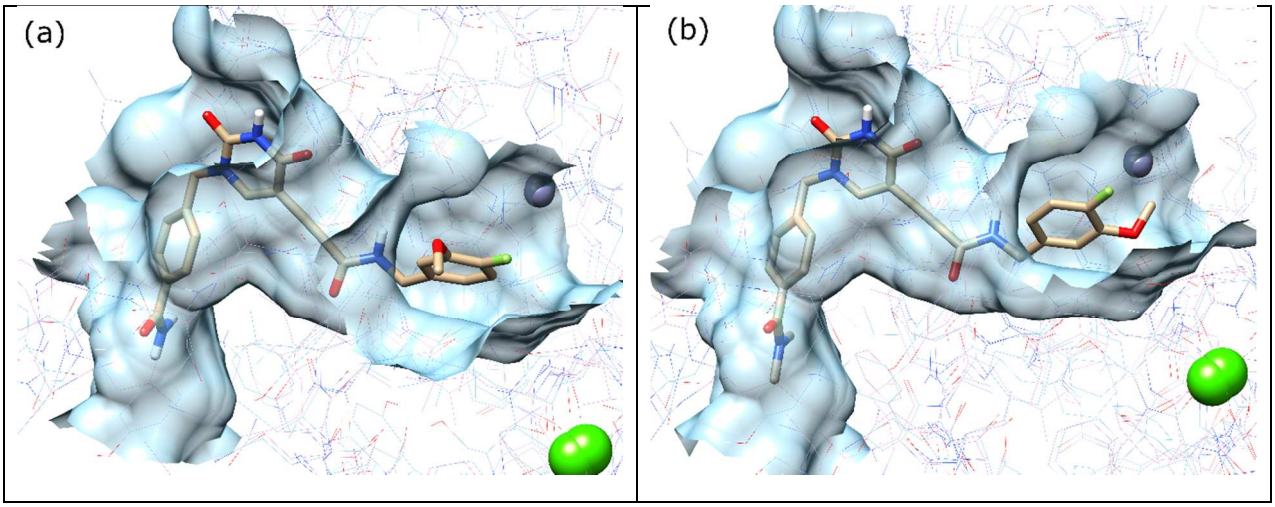


Figure 2. Initial design iteration towards a dual MMP-10/-13 inhibitor: (a) primary amide **3** and (b) dimethylated amide **5** docked into the overlay of the MMP-10 catalytic domain (PDB code: 1Q3A[33]) and MMP-13 catalytic domain (PDB code: 456C[34]); green spheres: calcium ions, purple spheres: zinc ions.

Figure 3 shows the design principle for **2**. Among the different docking poses of **2**, no potential binding to the zinc ion was observed as the carboxylic acid functional groups have been removed from **1** to avoid non-selective interference with metalloenzymes.

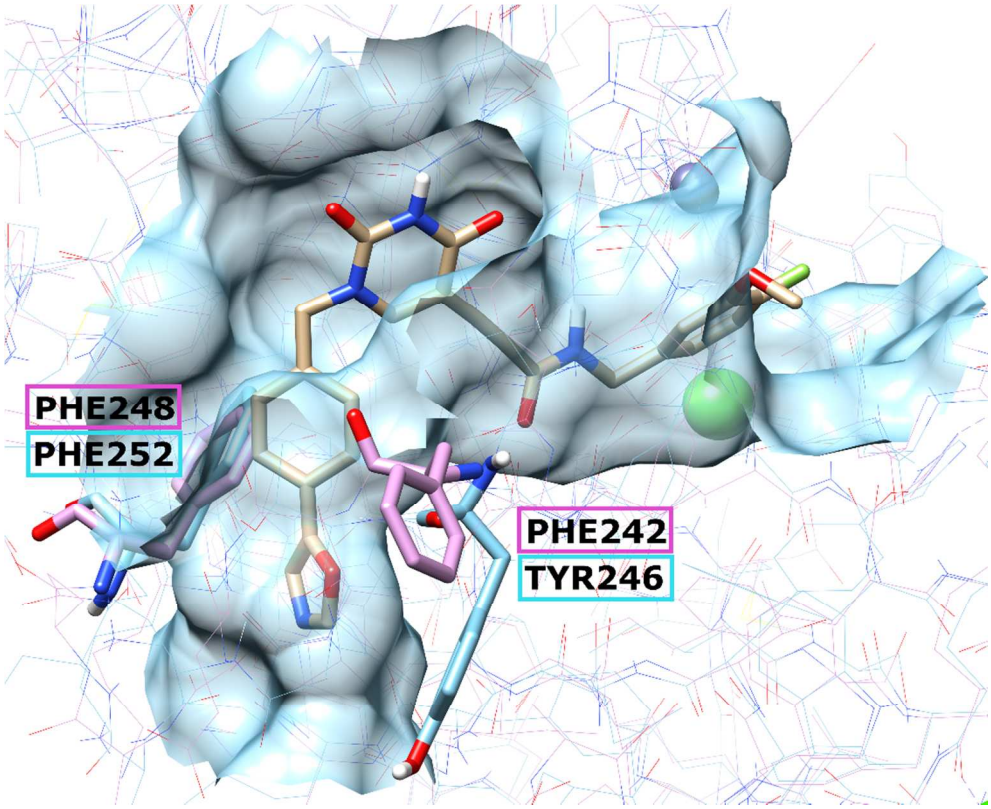


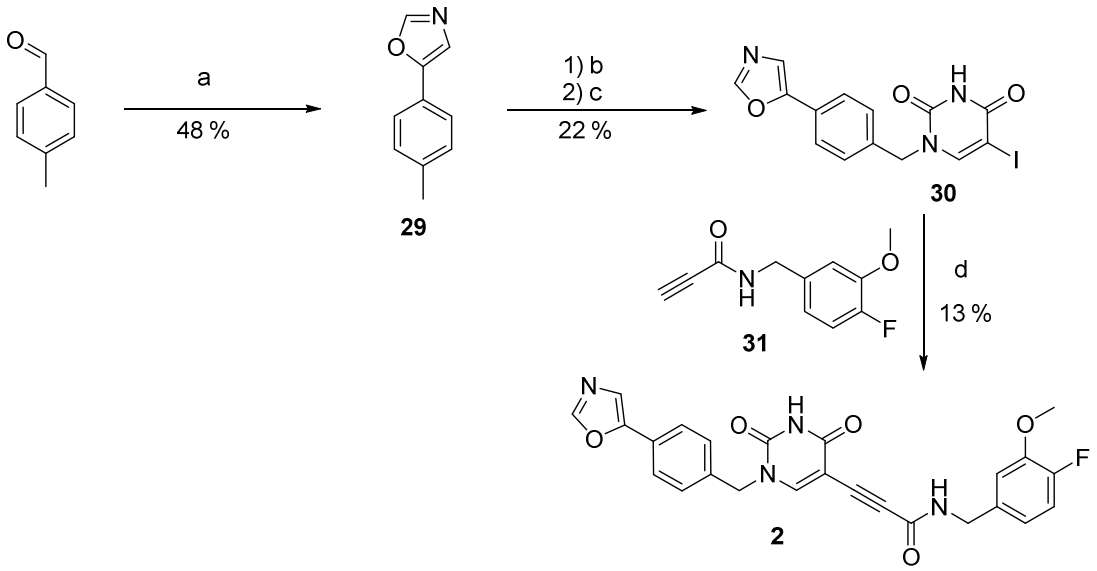
Figure 3. Design principle for **2** in the S1' sub site reaching into the aromatic S1'* pocket of the MMP-10 catalytic domain (purple chain, PDB code: 1Q3A[33]) and MMP-13 catalytic domain (blue chain, PDB code: 456C[34]). Aromatic interactions with the aromatic amino acid residues PHE242 and PHE248 of MMP-10 as well as TYR246 and PHE252 of MMP-13 are highlighted; green spheres: calcium ions, purple spheres: zinc ions.

Both proteins possess two aromatic amino acid residues in the S1'/S1'* pocket (PHE242 and PHE248 in MMP-10, and TYR246 and PHE252 in MMP-13), which can interact with the oxazole fragment of **2**. The analysis of a related crystal structure of MMP-10 (PDB code 3V96[35])

in complex with a tissue inhibitor of metalloproteinase (TIMP) in comparison to the used 1Q3A[33] structure highlighted differences within the specificity loop (S1' and S1'* pockets).[35] The flexibility in this part of the protein is limited to the amino acids 239-247 of the MMP-10 sequence. PHE242 is therefore considered to occupy an analogous position compared to the TYR246 residue in the MMP-13 structure.

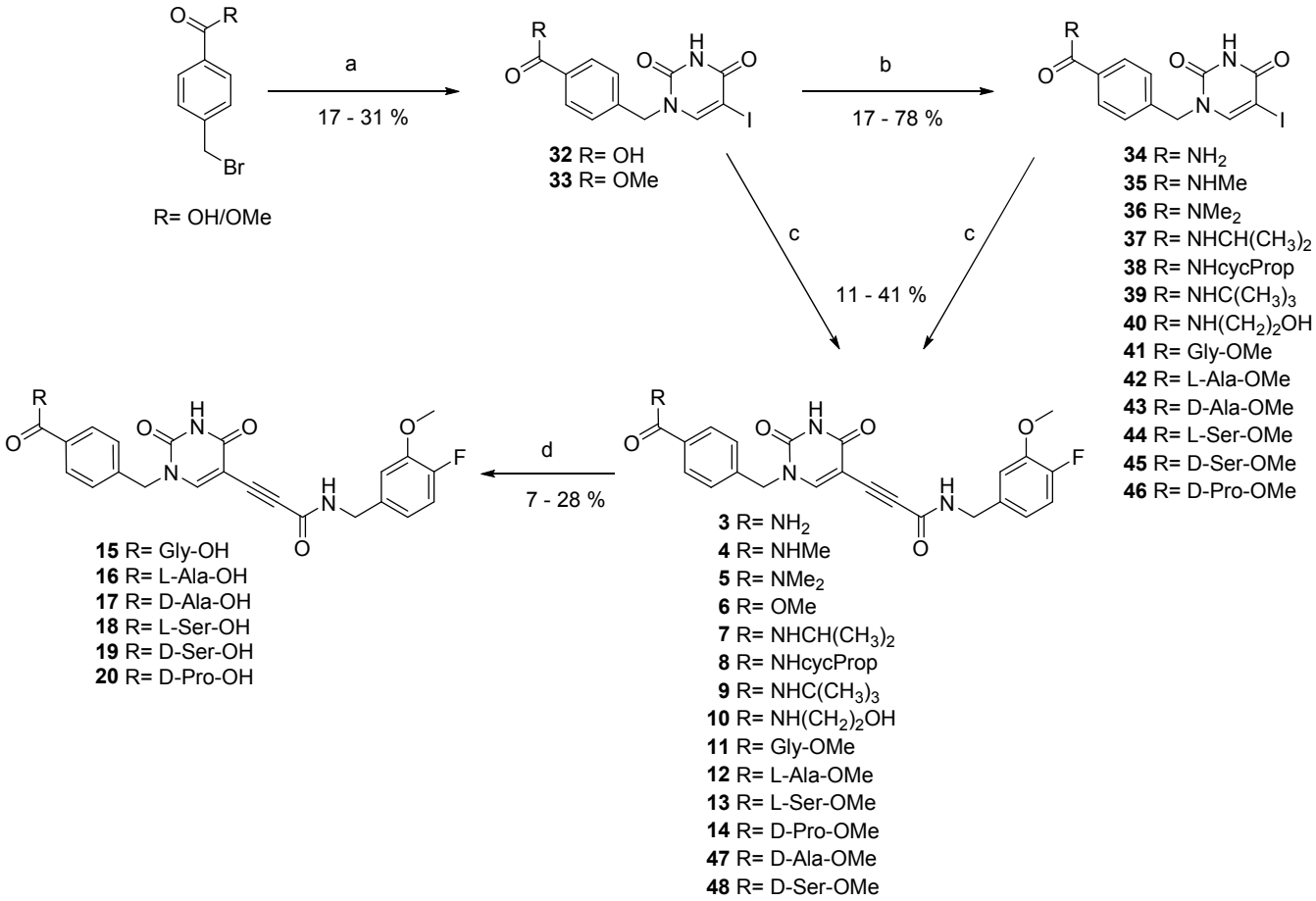
Chemistry. Schemes 1 to 3 show the synthetic procedures for the preparation of compounds **2-28**, which were tested for their potency towards MMP-10 and MMP-13. The synthesis of **2** was realized by a four steps synthesis. Starting with the formation of the oxazole **29**, followed by radical bromination and then substitution of the inserted bromine with 5-iodouracil furnished the building block **30** for the *Sonogashira* coupling in the final step to yield compound **2** (Scheme 1).

Scheme 1. Synthesis procedure for the dual MMP-10/-13 inhibitor **2**: (a) TOSMIC, K_2CO_3, MeOH, 80 °C, 2 h; (b) NBS, AIBN, CCl_4, reflux, 16 h; (c) 5-iodouracil, Cs_2CO_3, DMF, rt, 2.5 h; (d) $Pd(PPh_3)_4$, CuI, NEt_3, DMF, rt, 3 h.



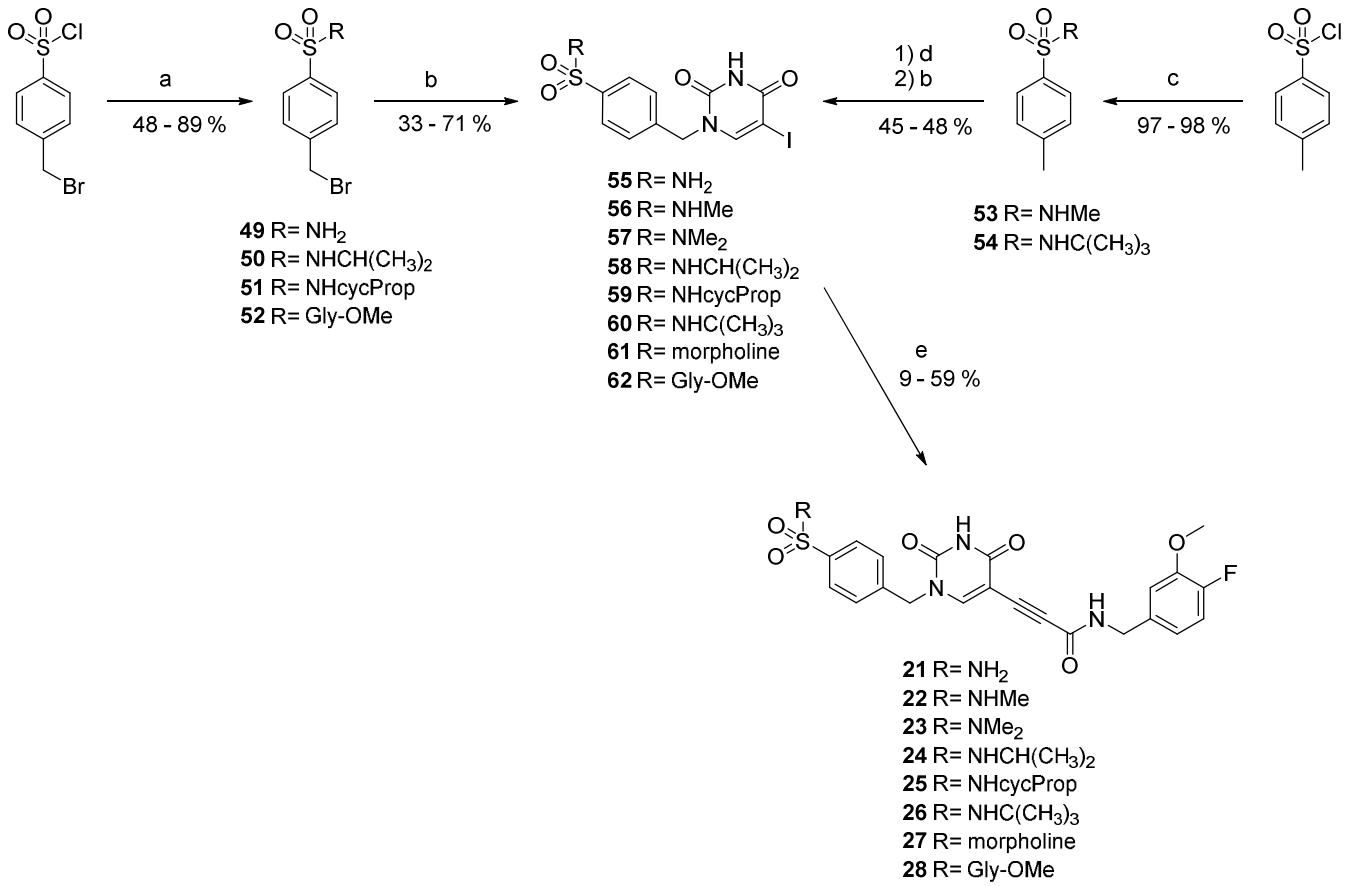
The carboxamide derivatives were synthesized starting with the substitution of the bromine of 4-(bromomethyl)benzoic acid by 5-iodouracil leading to compounds **32** and **33**, followed by amide coupling reactions to obtain intermediates **34-46**. The test compounds **3**-**14** and intermediates **47-48** were afforded by *Sonogashira* coupling, subsequent ester hydrolysis yielded the products **15-20** (Scheme 2).

Scheme 2. Synthesis procedure for the carboxamide derivatives: (a) 5-iodouracil, Cs_2CO_3, DMF, rt, 2.5 h; (b) R-NH_2, COMU, DIPEA, rt, 16 h; (c) **31**, $Pd(PPh_3)_4$, CuI, NEt_3, DMF, rt, 3 h; (d) KOH 3N, H_2O, rt, 2 h.



For the synthesis of the sulfonamide derivatives, two different entries were chosen. Both started with sulfonyl chlorides to yield the corresponding amides **49-52** and **53**, **54**, respectively. After the introduction of the 5-iodouracil fragment, the intermediates **55-62** were converted by *Sonogashira* coupling reactions to the final test compounds **21-28** (Scheme 3).

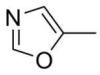
Scheme 3. Synthesis procedure for the sulfonamide derivatives: (a) R-NH_2, MeOH or THF, -78 °C to 0 °C, 2 h; (b) 5-iodouracil, Cs_2CO_3, DMF, rt, 2.5 h; (c) R^1-NH_2, THF, rt, 0.25 h; (d) NBS, DBPO, CCl_4, reflux, 16 h; (e) **31**, $Pd(PPh_3)_4$, CuI, NEt_3, DMF, rt, 3 h. Precursors for compounds **57** and **61** were commercially available: 4-(bromomethyl)-*N,N*-dimethylbenzenesulfonamide, R = NMe_2 for **57** and 4-[4-(bromomethyl)benzenesulfonyl]morpholine, R = morpholine for **61**.

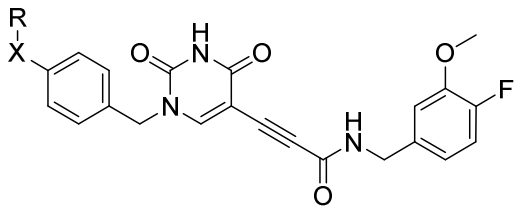


The biological evaluation of the synthesized compounds **2-28** was conducted at Reaction Biology Corporation, Malvern, PA, USA in a fluorescence assay using the (5-FAM/QXL™) FRET peptide as substrate (sequence = QXL® 520 - γ - Abu - Pro - Cha - Abu - Smc - His - Ala - Dab(5 - FAM) - Ala - Lys - NH_2 (Abu = 2-aminobutyric acid, Cha = β-cyclohexylalanine, Dab = diaminobutyric acid, Smc = S-methyl-L-cysteine, QXL® 520 = quencher, 5-FAM = 5-carboxyfluorescein, fluorescence dye). IC_{50} values and single dose residual activities were measured at least in duplicates with a starting concentration of 10 respectively 100 µM with 3 fold dilution for IC_{50} values and at 10 µM for single dose measurements. IC_{50} values were calculated using a four parameter least squares fit.

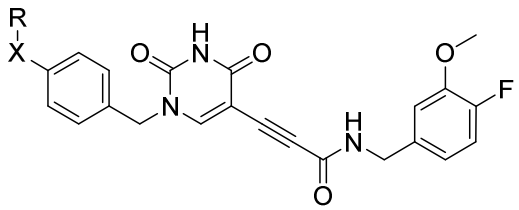
Structure-activity relationship. The resulting structure-activity relationship (SAR) showed a clear trend within the sulfonamide scaffold, as the affinity for MMP-10 is rising with more lipophilic amide residues, whereas the affinity for MMP-13 is kept at the same level (Table 1). Not only for the sulfonamide scaffold but also for the carboxamide scaffold the affinity for MMP-10 could be improved, while keeping the affinity for MMP-13 on a high level. IC_{50} values were determined for compounds showing less than 20 % residual enzyme activity and are listed in table 2. The bioisosteric replacement of the carboxamide function with an oxazole (**2**) provided the key entry for pi-pi-interactions with both MMP-10 and MMP-13 in order to reach a low nanomolar affinity for both target enzymes.

Table 1. Structure-activity relationship of the synthesized compounds **2-28**. Single dose measurements were carried out at an inhibitor concentration of 10 µM in duplicates. IC_{50} values were determined for compounds showing less than 20 % residual enzyme activity and are given in table 2.

	X	R	Residual activity [%]	
			MMP-10	MMP-13
2			2.8	0.4
3	C=O	NH_2	13.9	4.4
4	C=O	NHMe	15.4	7.5
5	C=O	NMe_2	7.1	1.4
6	C=O	OMe	18.0	4.6
7	C=O	$NHCH(CH_3)_2$	56.6	14.2
8	C=O	NHcycProp	30.8	5.9
9	C=O	$NHC(CH_3)_3$	55.7	13.3



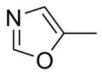
	X	R	Residual activity [%]	
			MMP-10	MMP-13
10	C=O	$NH(CH_2)_2OH$	14.2	2.6
11	C=O	Gly-OMe	52.8	12.2
12	C=O	L-Ala-OMe	15.5	2.5
13	C=O	L-Ser-OMe	21.9	3.9
14	C=O	D-Pro-OMe	17.5	1.8
15	C=O	Gly-OH	68.6	5.12
16	C=O	L-Ala-OH	28.5	0.8
17	C=O	D-Ala-OH	26.6	0.4
18	C=O	L-Ser-OH	10.4	0.8

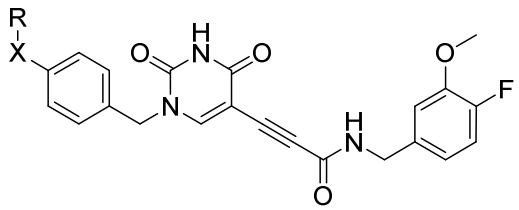


	X	R	Residual activity [%]	
			MMP-10	MMP-13
19	C=O	D-Ser-OH	77.0	5.6
20	C=O	D-Pro-OH	68.9	1.9
21	$S(=O)_2$	NH_2	19.1	3.9
22	$S(=O)_2$	NHMe	12.8	1.8
23	$S(=O)_2$	NMe_2	47.9	5.8
24	$S(=O)_2$	$NHCH(CH_3)_2$	10.2	2.2
25	$S(=O)_2$	NHcycProp	19.6	2.2
26	$S(=O)_2$	$NHC(CH_3)_3$	6.9	1.7
27	$S(=O)_2$	morpholine	15.7	1.2

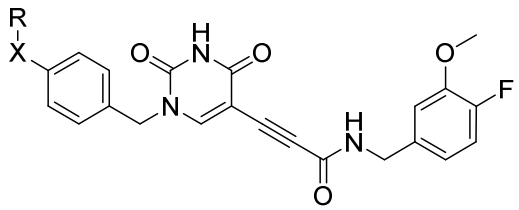
	X	R	Residual activity [%]	
			MMP-10	MMP-13
28	$S(=O)_2$	Gly-OMe	14.3	2.1

Table 2. Structure-activity relationship of compounds showing less than 20 % residual enzyme activity at an inhibitor concentration of 10 μM. IC_{50} values are given with their corresponding 95 % confidence interval of pIC_{50}.

	X	R	IC_{50} (MMP-10) [nM]		IC_{50} (MMP-13) [nM]	
2			31	[pIC_{50} = 7.61-7.53]	5	[pIC_{50} = 8.44-8.26]
3	C=O	NH_2	1210	[pIC_{50} = 5.32-4.74]	111	[pIC_{50} = 7.11-6.79]



	X	R	IC_{50} (MMP-10) [nM]		IC_{50} (MMP-13) [nM]	
4	C=O	NHMe	635	[pIC_{50} = 6.32-6.08]	86	[pIC_{50} = 7.19-6.95]
5	C=O	NMe_2	243	[pIC_{50} = 6.73-6.23]	89	[pIC_{50} = 7.15-6.96]
6	C=O	OMe	506	[pIC_{50} = 6.34-6.25]	128	[pIC_{50} = 7.00-6.80]
10	C=O	$NH(CH_2)_2OH$	347	[pIC_{50} = 6.56-6.40]	58	[pIC_{50} = 7.35-7.14]
12	C=O	L-Ala-OMe	751	[pIC_{50} = 6.31-5.97]	149	[pIC_{50} = 6.99-6.71]
14	C=O	D-Pro-OMe	2050	[pIC_{50} = 5.90-5.31]	175	[pIC50 = 6.88-6.66]
18	C=O	L-Ser-OH	705	[pIC_{50} = 6.30-6.08]	14	[pIC_{50} = 8.11-7.82]
21	$S(=O)_2$	NH_2	7380	[pIC_{50} = 5.32-4.74]	258	[pIC_{50} = 6.69-6.49]
22	$S(=O)_2$	NHMe	531	[pIC_{50} = 6.39-6.17]	35	[pIC_{50} = 7.59-7.39]
24	$S(=O)_2$	$NHCH(CH_3)_2$	147	[pIC_{50} = 7.17-6.85]	25	[pIC_{50} = 7.82-7.46]



	X	R	IC_{50} (MMP-10) [nM]		IC_{50} (MMP-13) [nM]	
25	$S(=O)_2$	NHcycProp	110	[pIC_{50} = 7.17-6.95]	14	[pIC_{50} = 7.99-7.93]
26	$S(=O)_2$	$NHC(CH_3)_3$	131	[pIC_{50} = 7.29-6.85]	26	[pIC_{50} = 7.92-7.68]
27	$S(=O)_2$	morpholine	1930	[pIC_{50} = 6.12-5.77]	66	[pIC_{50} = 7.79-7.44]
28	$S(=O)_2$	Gly-OMe	485	[pIC_{50} = 6.61-6.36]	32	[pIC_{50} = 7.82-7.46]

The most potent non-hydroxamate MMP-10 inhibitor found in literature was originally developed as a very potent MMP-13 inhibitor.[24] This compound, **T-26C** (structure given in the supporting information), was disclosed with an IC_{50} value of 160 nM for MMP-10 and 6.9 pM for MMP-13. In our assay system, values of 358 nM for MMP-10 [pIC50 = 6.51-6.39] and 0.19 nM for MMP-13 [pIC50 = 9.86-9.56] were measured. The dual MMP-10/-13 inhibitor **2** displays a ten times lower IC_{50} value making it, to the best of our knowledge, the most potent non-hydroxamate MMP-10 inhibitor currently known. Within the MMP family, the selectivity profile of **2** was determined (MMP-1, -2, -3, -7, -8, -9, -12 and -14). Except for MMP-3, the remaining enzyme activity was >85 % at an inhibitor concentration of 10 μM for all of the tested enzymes, which demonstrates the high selectivity for MMP-10/-13 (Table 3). The determination of the

IC_{50} value of **2** on MMP-3 (2.91 μM [pIC_{50} = 6.01-4.78]) demonstrates high selectivity for MMP-10/-13, even between the two structurally closely related stromelysins MMP-3 and MMP-10.[36]

Table 3. Inhibition profile of **2** at an inhibitor concentration of 10 μM. Residual enzyme activity is given at an inhibitor concentration of 10 μM (measured in duplicate).

	Remaining activity [%]							
	MMP							
	-1	-2	-3	-7	-8	-9	-12	-14
2	100	100	37.3 ± 2.1	87.1 ± 1.2	96.7 ± 2.9	97.8 ± 0.4	88.5 ± 0.6	97.6 ± 0.7

Taking into account that both MMPs are validated drug targets, this dual inhibitor represents a promising base for polypharmacological approaches in complex systems.[37] Literature evidence demonstrates the mutual relevance of the two targeted proteases.[20,22,23] By using **2**, this interconnection can be examined in complex diseases related to these proteins. MMP-10 was shown to activate the pro-collagenases and thereby to promote collagenolytic activity.[20,22] The here presented dual inhibitor **2** is able to inhibit MMP-10 and thereby can reduce the collagenolytic activity. In addition, the inhibition of MMP-13 can block one of the collagenases selectively for the time of administration.

CONCLUSION

In conclusion, we succeeded in the parallel optimization of a dual MMP-10/-13 inhibitor to discover the non-hydroxamate inhibitor **2** with outstanding affinity for MMP-10 and similar affinity for MMP-13. Selectivity for these two proteins among the MMP family was additionally shown. The benefit of a dual MMP inhibitor with a clean inhibition profile is based on the network like organization of MMPs, in which specific proteases activate each other and take related functions in this complex system. Inhibitor **2** represents a viable tool compound to gain further insight into this complex system, and provides an excellent starting point for the development of therapies based on targeted polypharmacology.

EXPERIMENTAL SECTION

Chemistry General Procedures. All reagents and solvents were purchased from Sigma Aldrich, TCI or Fluorochem and used as received. All NMR spectra were recorded on a Bruker AVANCE III HD 500 One Bay spectrometer with a magnetic field of 11.75 T and a 5mm SmartProbe BB(F)-H-D. For 1H NMR spectra, a frequency of 500 MHz resulted. Chemical shifts are reported in ppm from tetramethylsilane as internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint. = quintet, br = broad, m = multiplet), coupling constants (Hz), integration. For ^{13}C NMR spectra, a frequency of 125 MHz resulted. Chemical shifts are reported in ppm from tetramethylsilane as internal standard. High-resolution mass spectrometry was performed on an Agilent Technologies 6530 Q-TOF. Purity was assayed by HPLC (Interchim Strategy C-18 column, 4.6 mm × 250 mm) with a gradient of 5 – 100 % methanol in 0.2 % aqueous acetic acid with UV detection at λ = 254 nm.

All final compounds were obtained with ≥95 % purity. Microwave synthesis was performed in a Biotage initiator.

General procedure for the nucleophilic substitution by 5-iodouracil. The 4-(bromomethyl)benzoic acid derivative (4.7 mmol, 1.0 eq) was dissolved in DMF (7 mL) and added dropwise to a suspension of 5-iodouracil (8.4 mmol, 1.8 eq) and caesium carbonate (5.7 mmol, 1.2 eq) in DMF (18 mL), which has been stirred at room temperature for 15 minutes before under nitrogen atmosphere. After the complete addition, the mixture was stirred for 2.5 h. The mixture was then separated between ethyl acetate and 2 M hydrochloric acid. After concentration, the resulting liquid was purified by flash chromatography (reversed phase C18, gradient: 10 – 100% methanol in water).

General procedure for the amide formation. Procedure A. Carboxylic acid (0.17 mmol), amine (0.45 mmol), COMU (0.17 mmol) and diisopropylethylamine (DIPEA, 0.1 mL) were dissolved in DMF (1.5 mL), and the yellow solution was stirred over night at room temperature under nitrogen atmosphere. The reaction mixture was then separated between ethyl acetate and 2 M hydrochloric acid, concentrated and then purified by flash chromatography (reversed phase C18, gradient: 10 – 100% methanol in water). **Procedure B.** Sulfonyl chloride (2.78 mmol, 1.5 eq) was dissolved in THF or methanol (20 mL) and cooled. At this temperature, the amine (1.86 mmol, 1.0 eq) was added dropwise and the mixture was stirred for 30 min. Then, 6 M hydrochloric acid was added to the cold solution, which was then allowed to warm up to room temperature. The mixture was then concentrated in vacuo and purified by flash chromatography (SiO_2, gradient: dichloromethane – methanol).

General procedure for the *Sonogashira* coupling. Iodouracil derivative (0.10 mmol, 1.0 eq), alkyne (0.17 mmol, 1.7 eq), tetrakistriphenylphosphine palladium (0.01 mmol, 0.1 eq), copper

iodide (0.05 mmol, 0.5 eq) and triethylamine (0.2 mL) were dissolved under argon atmosphere in DMF (1.5 mL), and stirred at room temperature for 3 h. The reaction mixture was separated between ethyl acetate and 2 M hydrochloric acid, concentrated and purified by flash chromatography (reversed phase C18, gradient: 10 % – 100% methanol in water).

General procedure for the ester hydrolysis. The ester was suspended in potassium hydroxide solution (3M, 3 mL) and stirred at room temperature for 2 h. The reaction mixture was then acidified to pH 1 with 6 M hydrochloric acid and extracted with ethyl acetate. The combined organic layers were concentrated under reduced pressure und purified by flash chromatography.

***N*-[(4-Fluoro-3-methoxyphenyl)methyl]-3-(1-{[4-(1,3-oxazol-5-yl)phenyl]methyl}-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)prop-2-ynamide (2)** was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **30** and **31**. Before flash chromatography, the product was washed with 10 ml of a 1/1 mixture of methanol/water. **2** was obtained with 13 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.80 (br s, 1H), 9.15 (t, J = 6.0 Hz, 1H), 8.44 (s, 1H), 8.43 (s, 1H), 7.74-7.70 (m, 2H), 7.69 (s, 1H), 7.46-7.41 (m, 2H), 7.14 (dd, J = 11.9 Hz, 8.5 Hz, 1H), 7.07 (dd, J = 8.5 Hz, 2.0 Hz, 1H), 6.80 (ddd, J = 8.2 Hz, 4.2 Hz, 2.0 Hz, 1H), 4.94 (s, 2H), 4.27 (d, J = 6.0 Hz, 2H), 3.81 (s, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 161.9, 152.2, 151.9, 151.5, 150.6 (d, J_{CF} = 242.0 Hz), 150.2, 150.1, 146.8 (d, J_{CF} = 10.9 Hz), 136.7, 135.5 (d, J_{CF} = 3.4 Hz), 128.5 (2C), 126.9, 124.4 (2C), 122.2, 119.5 (d, J_{CF} = 7.7 Hz), 115.5 (d, J_{CF} = 18.0 Hz), 113.1 (d, J_{CF} = 1.6 Hz), 95.5, 85.8, 77.8, 55.9, 50.8, 42.0 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{25}H_{19}FN_4O_5$: 474.134, found: 474.142.

4-{[5-(2-{[(4-Fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}benzamide (3) was synthesized according to the general

procedure of the *Sonogashira* coupling from intermediates **34** and **31**. Before flash chromatography, the product was washed with 10 ml of a 1/1 mixture of methanol/water. **3** was obtained with 20 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.83 (s, 1H), 9.14 (t, J = 6.2 Hz, 1H), 8.38 (s, 1H), 7.96 (s, 1H), 7.87-7.81 (m, 2H), 7.42-7.36 (m, 2H), 7.36 (s, 1H), 7.15 (dd, J = 11.5 Hz, 8.4 Hz, 1H), 7.08 (dd, J = 8.4 Hz, 1.9 Hz, 1H), 6.81 (ddd, J = 8.3 Hz, 4.6 Hz, 2.2 Hz, 1H), 4.95 (s, 2H), 4.28 (d, J = 6.2 Hz, 2H), 3.82 (s, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 167.9 (2C), 152.8, 151.9, 151.8, 151.0 (d, J_{CF} = 241.0 Hz), 147.3 (d, J_{CF} = 10.6 Hz), 140.0, 136.0 (d, J_{CF} = 4.0 Hz), 134.2, 128.3 (2C), 127.8 (2C), 120.0 (d, J_{CF} = 6.7 Hz), 116.0 (d, J_{CF} = 17.9 Hz), 113.6 (d, J_{CF} = 1.6 Hz), 96.0, 87.2, 56.4, 51.3, 42.5 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{23}H_{19}FN_4O_5$: 450.134, found: 450.141.

4-{[5-(2-{[(4-Fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}-*N*-methylbenzamide (4) was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **35** and **31**. Before flash chromatography, the product was washed with 10 ml of a 1/1 mixture of methanol/water. **4** was obtained with 22 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.83 (s, 1H), 9.16 (t, J = 6.2 Hz, 1H), 8.43 (s, 1H), 8.42 (q, J = 4.5 Hz, 1H), 7.84-7.77 (m, 2H), 7.43-7.35 (m, 2H), 7.15 (dd, J = 11.7 Hz, 8.5 Hz, 1H), 7.08 (dd, J = 8.5 Hz, 2.0 Hz, 1H), 6.81 (ddd, J = 8.0 Hz, 4.5 Hz, 2.0 Hz, 1H), 4.97 (s, 2H), 4.28 (d, J = 6.0 Hz, 2H), 8.32 (s, 3H), 2.78 (d, J = 4.5 Hz, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 166.7, 162.3, 152.7, 152.0, 151.1 (d, J_{CF} = 243.6 Hz), 150.5, 147.3 (d, J_{CF} = 9.3 Hz), 139.6, 135.9 (d, J_{CF} = 4.1 Hz), 134.5, 127.8 (4C), 120.0 (d, J_{CF} = 6.9 Hz), 116.0 (d, J_{CF} = 17.9 Hz), 113.6 (d, J_{CF} = 1.5 Hz), 96.0, 87.3, 78.3, 56.4, 51.3, 42.5, 26.7 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{24}H_{21}FN_4O_5$: 464.150, found: 464.158.

4-{[5-(2-{[(4-Fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}-*N,N*-dimethylbenzamide (5) was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **36** and **31**. Before flash chromatography, the product was washed with 10 ml of a 1/1 mixture of methanol/water. **5** was obtained with 28 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.83 (s, 1H), 9.14 (t, J = 6.3 Hz, 1H), 8.41 (s, 1H), 7.42-7.35 (m, 4H), 7.15 (dd, J = 11.6 Hz, 8.6 Hz, 1H), 7.08 (dd, J = 8.6 Hz, 2.0 Hz, 1H), 6.81 (ddd, J = 8.3 Hz, 4.3 Hz, 2.0 Hz, 1H), 4.95 (s, 2H), 4.28 (d, J = 6.3 Hz, 2H), 3.82 (s, 3H), 2.97 (s, 3H), 2.89 (s, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 170.2, 163.0, 151.9, 151.0, 151.0 (d, J_{CF} = 241.4 Hz), 147.3 (d, J_{CF} = 10.3 Hz), 138.1, 136.4, 136.0 (d, J_{CF} = 2.5 Hz), 127.8 (2C), 127.8 (2C), 120.0 (d, J_{CF} = 6.5 Hz), 116.0 (d, J_{CF} = 18.8 Hz), 113.6 (d, J_{CF} = 2.0 Hz), 96.0), 87.7, 78.7, 56.4, 51.2, 42.5, 39.5, 35.2 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{25}H_{23}FN_4O_5$: 478.165, found: 478.174.

Methyl 4-{[5-(2-{[(4-fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}benzoate (6) was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **33** and **31**. Before flash chromatography, the product was washed with 10 ml of a 1/1 mixture of methanol/water. **6** was obtained with 11 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.85 (br s, 1H), 9.16 (t, J = 6.0 Hz, 1H), 8.45 (s, 1H), 7.97-7.91 (m, 2H), 7.48-7.41 (m, 2H), 7.15 (dd, J = 11.6 Hz, 8.2 Hz, 1H), 7.07 (dd, J = 8.4 Hz, 1.9 Hz, 1H), 6.80 (ddd, J = 8.2 Hz, 4.2 Hz, 1.9 Hz, 1H), 5.00 (s, 2H), 4.27 (d, J = 6.1 Hz, 2H), 3.85 (s, 3H), 3.82 (s, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 166.4, 153.1, 152.1, 151.1 (d, J_{CF} = 245.4 Hz), 147.3 (d, J_{CF} = 9.2 Hz), 142.1, 135.9 (d, J_{CF} = 2.6 Hz), 130.0 (2C), 129.5, 129.3, 129.2, 128.1 (2C), 120.0 (d, J_{CF}

= 6.0 Hz), 116.0 (d, J_{CF} = 17.4 Hz), 113.6 (d, J_{CF} = 1.1 Hz), 96.1, 87.4, 78.5, 56.4, 52.7, 51.4, 42.5 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{24}H_{20}FN_3O_6$: 465.134, found: 465.141.

4-{[5-(2-{[(4-Fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}-*N*-(propan-2-yl)benzamide (7) was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **37** and **31**. Before flash chromatography, the product was washed with 10 ml of a 1/1 mixture of methanol/water. **7** was obtained with 12 % yield as a white solid. ^{1}H NMR (500 MHz, DMF-d_7, 25 °C, TMS): δ 11.79 (br s, 1H), 9.23 (t, *J* = 6.7 Hz, 1H), 8.48 (s, 1H), 8.21 (d, *J* = 7.4 Hz, 1H), 7.98-7.92 (m, 2H), 7.55-7.48 (m, 2H), 7.20 (dd, *J* = 8.4 Hz, 1.6 Hz, 1H), 7.17 (dd, *J* = 11.8 Hz, 8.4 Hz, 1H), 6.92 (ddd, *J* = 7.8 Hz, 4.1 Hz, 1.9 Hz, 1H), 5.11 (s, 2H), 4.42 (d, *J* = 6.2 Hz, 2H), 4.20 (octett, 7.0 Hz, 1H), 3.89 (s, 3H), 1.22 (d, *J* = 6.7 Hz, 6H) ppm. ^{13}C NMR (125 MHz, DMF-d_7, 25 °C, TMS): δ 166.4, 163.7, 153.9, 152.6, 152.3 (d, J_{CF} = 243.8Hz), 151.8, 148.5 (d, J_{CF} = 10.3 Hz), 140.6, 137.0 (d, J_{CF} = 3.6 Hz), 136.0, 128.7 (4C), 120.8 (d, J_{CF} = 7.6 Hz), 116.6 (d, J_{CF} = 18.6 Hz), 114.5 (d, J_{CF} = 2.0 Hz), 97.3, 88.0, 78.8, 56.8, 52.2, 43.5, 42.5, 23.0 (2C) ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{26}H_{25}FN_4O_5$: 492.181, found: 492.188.

***N*-Cyclopropyl-4-{[5-(2-{[(4-fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}benzamide (8)** was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **38** and **31**. Before flash chromatography, the product was washed with 10 ml of a 1/1 mixture of methanol/water. **8** was obtained with 17 % yield as a white solid. ^{1}H NMR (500 MHz, DMF-d_7, 25 °C, TMS): δ 11.65 (br s, 1H), 9.41 (t, *J* = 5.8 Hz, 1H), 8.770 (s, 1H), 8.63 (d, *J* = 4.0 Hz, 1H), 8.14-8.08 (m, 2H), 7.74-7.68 (m, 2H), 7.38 (dd, *J* = 8.5 Hz, 1.6 Hz, 1H), 7.34 (dd, *J* = 11.8 Hz, 8.5 Hz, 1H), 7.09 (ddd, *J* = 8.1 Hz, 4.3 Hz, 2.0 Hz, 1H), 5.29 (s, 2H), 4.60 (d, *J* = 6.1 Hz, 2H), 4.07 (s, 3H), 1.54-

1.43 (m, 1H), 0.97-0.88 (m, 2H), 0.86-0.78 (m, 2H) ppm. ^{13}C NMR (125 MHz, DMF-d_7, 25 °C, TMS): δ 168.4, 163.7, 162.8, 153.8, 152.8, 152.3 (d, J_{CF} = 243.2 Hz), 151.4, 148.5 (d, J_{CF} = 10.7 Hz), 140.5, 137.0 (d, J_{CF} = 3.2 Hz), 135.6, 128.8 (2C), 128.7 (2C), 120.8 (d, J_{CF} = 6.9 Hz), 116.6 (d, J_{CF} = 18.2 Hz), 114.5 (d, J_{CF} = 1.8Hz), 97.3, 88.1, 78.4, 56.8, 52.3, 43.5, 24.2, 6.5 (2C) ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{26}H_{23}FN_4O_5$: 490.165, found: 490.173.

***N*-tert-Butyl-4-{[5-(2-{[(4-fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}benzamide (9)** was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **39** and **31**. Before flash chromatography, the product was washed with 10 ml of a 1/1 mixture of methanol/water. **9** was obtained with 40 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.82 (br s, 1H), 9.14 (t, *J* = 5.7 Hz, 1H), 8.39 (s, 1H), 7.77-7.73 (m, 2H), 7.72 (s, 1H), 7.40-7.33 (m, 2H), 7.14 (dd, *J* = 11.6 Hz, 8.2 Hz, 1H), 7.07 (dd, *J* = 8.5 Hz, 2.0 Hz, 1H), 6.80 (ddd, *J* = 8.3 Hz, 4.4 Hz, 2.1 Hz, 1H), 4.95 (s, 2H), 4.27 (d, *J* = 6.1 Hz, 2H), 3.82 (s, 3H), 1.36 (s, 9H) ppm. ^{13}C NMR (125 MHz, DMF-d_7, 25 °C, TMS): δ 166.3, 162.7, 152.9, 151.4, 151.3, 151.2 (d, J_{CF} = 240.4 Hz), 147.5 (d, J_{CF} = 11.1 Hz), 139.5, 136.0 (d, J_{CF} = 3.9 Hz), 136.0, 127.7 (2C), 127.6 (2C), 119.8 (d, J_{CF} = 5.7 Hz), 115.6 (d, J_{CF} = 18.7 Hz), 113.4 (d, J_{CF} = 1.1 Hz), 96.2, 85.9, 78.3, 55.8, 51.2, 51.1, 42.5, 28.3 (3C) ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{27}H_{27}FN_4O_5$: 506.197, found: 506.201.

4-{[5-(2-{[(4-Fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}-*N*-(2-hydroxyethyl)benzamide (10) was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **40** and **31**. Before flash chromatography, the product was washed with 10 ml of a 1/1 mixture of methanol/water. **10** was obtained with 13 % yield as a white solid. ^{1}H NMR (500 MHz, DMF-d_7,

25 °C, TMS): δ 11.84 (br s, 1H), 9.24 (t, *J* = 6.3 Hz, 1H), 8.56-8.49 (m, 2H), 8.01-7.96 (m, 2H), 7.57-7.51 (m, 2H), 7.20 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H), 7.17 (dd, *J* = 11.6 Hz, 8.4 Hz, 1H), 6.92 (ddd, *J* = 8.3 Hz, 4.3 Hz, 2.1 Hz, 1H), 5.12 (s, 2H), 4.87 (d, *J* = 5.7 Hz, 1H), 4.42 (d, *J* = 6.1 Hz, 2H), 3.89 (s, 3H), 3.66 (q, *J* = 5.9 Hz, 2H), 3.47 (q, *J* = 5.9 Hz, 2H) ppm. ^{13}C NMR (125 MHz, DMF-d_7, 25 °C, TMS): δ 167.4, 162.8, 153.8, 152.8, 152.3 (d, J_{CF} = 243.6 Hz), 151.4, 148.5 (d, J_{CF} = 11.2 Hz), 140.5, 137.0 (d, J_{CF} = 3.0 Hz), 135.7, 128.8 (2C), 128.8 (2C), 120.8 (d, J_{CF} = 7.4 Hz), 116.6 (d, J_{CF} = 18.1 Hz), 114.5 (d, J_{CF} = 1.8 Hz), 97.3, 88.1, 78.4, 61.6, 56.8, 52.3, 43.8, 43.5 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{25}H_{23}FN_4O_5$: 494.160, found: 494.169.

Methyl 2-[(4-{[5-(2-{[(4-fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}phenyl)formamido]acetate (11) was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **41** and **31**. Before flash chromatography, the product was washed with 10 ml of a 1/1 mixture of methanol/water. **11** was obtained with 17 % yield as a white solid. ^{1}H NMR (500 MHz, DMF-d_7, 25 °C, TMS): δ 11.93 (br s, 1H), 9.28 (t, *J* = 6.1 Hz, 1H), 9.04 (t, *J* = 5.7 Hz, 1H), 8.56 (s, 1H), 8.05-8.00 (m, 2H), 7.63-7.57 (m, 2H), 7.24 (dd, *J* = 8.5 Hz, 1.9 Hz, 1H), 7.20 (dd, *J* = 11.6 Hz, 8.3 Hz, 1H), 6.95 (ddd, *J* = 8.3 Hz, 4.4 Hz, 2.1 Hz, 1H), 5.17 (s, 2H), 4.46 (d, *J* = 6.0 Hz, 2H), 4.18 (d, *J* = 5.8 Hz, 2H), 3.93 (s, 3H), 3.74 (s, 3H) ppm. ^{13}C NMR (125 MHz, DMF-d_7, 25 °C, TMS): δ 171.5, 167.6, 163.8, 158.7, 154.7 (d, J_{CF} = 244.4 Hz), 152.7, 151.3, 148.4 (d, J_{CF} = 10.6 Hz), 141.0, 136.8 (d, J_{CF} = 3.6 Hz), 134.7, 128.8 (2C), 128.7 (2C), 120.7 (d, J_{CF} = 6.0 Hz), 116.5 (d, J_{CF} = 17.4 Hz), 114.3 (d, J_{CF} = 2.5 Hz), 97.2, 88.0, 78.3, 56.7, 52.4, 52.2, 43.4, 42.3 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{26}H_{23}FN_4O_7$: 522.155, found: 522.161.

Methyl (2*S*)-2-[(4-{[5-(2-{[(4-fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}phenyl)formamido]propanoate (12) was

synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **42** and **31**. Before flash chromatography, the product was washed with 10 ml of a 1/1 mixture of methanol/water. **12** was obtained with 23 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.83 (s, 1H), 9.16 (t, J = 6.0 Hz, 1H), 8.79 (d, J = 6.8 Hz, 1H), 8.42 (s, H), 7.91-7.82 (m, 2H), 7.49-7.39 (m, 2H), 7.15 (dd, J = 11.6 Hz, 8.0 Hz, 1H), 7.08 (dd, J = 8.8 Hz, 2.0 Hz, 1H), 6.81 (ddd, J = 8.0 Hz, 4.4 Hz, 2.0 Hz, 1H), 4.98 (s, 2H), 4.48 (quint., J = 7.2 Hz, 1H), 4.28 (d, J = 6.0 Hz, 2H), 3.82 (s, 3H), 8.64 (s, 3H), 1.40 (d, J = 7.2 Hz, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 173.6, 166.3, 152.7, 152.0, 151.1 (d, J_{CF} = 242.0 Hz), 150.5, 147.3 (d, J_{CF} = 10.6 Hz), 140.1, 135.9 (d, J_{CF} = 3.0 Hz), 133.6, 128.3 (2C), 127.9 (2C), 120.0 (d, J_{CF} = 6.6 Hz), 116.0 (d, J_{CF} = 18.0 Hz), 113.6 (d, J_{CF} = 1.4 Hz), 96.0, 87.3, 78.2, 56.4, 52.4, 51.4, 48.7, 42.5, 17.2 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{27}H_{25}FN_4O_7$: 536.171, found: 536.177.

Methyl (2*S*)-2-[(4-{[5-(2-{[(4-fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}phenyl)formamido]-3-hydroxypropanoate (13) was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **44** and **31**. Before flash chromatography, the product was washed with 10 ml of a 1/1 mixture of methanol/water. **13** was obtained with 36 % yield as a white solid. ^{1}H NMR (500 MHz, DMF-d_7, 25 °C, TMS): δ 9.24 (t, J = 6.3 Hz, 1H), 8.83 (d, J = 7.19 Hz, 1H), 8.53 (s, 1H), 8.13-8.08 (m, 2H), 7.58-7.53 (m, 2H), 7.20 (dd, J = 8.4 Hz, 2.1 Hz, 1H), 7.17 (dd, J = 11.6 Hz, 8.4 Hz, 1H), 6.92 (ddd, J = 8.2 Hz, 4.5 Hz, 2.1 Hz, 1H), 5.13 (s, 2H), 4.69-4.64 (m, 1H), 4.42 (d, J = 6.1 Hz, 2H), 4.01-3.93 (m, 2H), 3.89 (s, 3H), 3.70 (s, 3H) ppm. ^{13}C NMR (125 MHz, DMF-d_7, 25 °C, TMS): δ 172.3, 167.5, 162.8, 153.8, 152.7, 151.4, 149.2 (d, J_{CF} = 345.8 Hz), 141.0, 137.0 (d, J_{CF} = 3.6 Hz), 134.8, 129.2 (2C), 129.1, 128.8 (2C), 120.8 (d, J_{CF} = 6.6 Hz), 116.6 (d,

J_{CF} = 18.7 Hz), 114.5 (d, J_{CF} = 1.8 Hz), 97.3, 88.1, 78.4, 62.7, 57.5, 56.8, 52.6, 52.3, 43.5 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{27}H_{25}FN_4O_8$: 552.166, found: 552.176.

Methyl (2*R*)-1-(4-{[5-(2-{[(4-fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}benzoyl)pyrrolidine-2-carboxylate (14) was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **46** and **31**. Before flash chromatography, the product was washed with 10 ml of a 1/1 mixture of methanol/water. **14** was obtained with 41 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.85 (s, 1H), 9.17 (t, *J* = 6.1 Hz, 1H), 8.47 (s, 1H), 7.58-7.49 (m, 2H), 7.44-7.38 (m, 2H), 7.15 (dd, *J* = 11.6 Hz, 8.4 Hz, 1H), 7.08 (dd, *J* = 8.4 Hz, 1.7 Hz, 1H), 6.81 (ddd, *J* = 8.1 Hz, 4.4 Hz, 1.7 Hz, 1H), 4.98 (s, 2H), 4.28 (d, *J* = 6.1 Hz, 2H), 4.10 (q, *J* = 5.2 Hz, 1H), 3.82 (s, 3H), 3.67 (s, 3H), 3.55-3.47 (m, 2H), 2.32-2.22 (m, 1H), 1.95-1.80 (m, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 172.8, 168.3, 162.1, 152.7, 152.1, 151.1 (d, J_{CF} = 242.9 Hz), 150.4, 147.3 (d, J_{CF} = 11.2 Hz), 138.7, 135.9 (d, J_{CF} = 4.3 Hz), 135.9, 128.0 (2C), 127.9 (2C), 120.0 (d, J_{CF} = 6.1 Hz), 116.0 (d, J_{CF} = 18.1 Hz), 113.6 (d, J_{CF} = 1.3 Hz), 96.0, 87.4, 78.1, 59.4, 56.4, 52.3, 51.3, 50.0, 42.5, 29.3, 25.5 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{29}H_{27}FN_4O_7$: 562.186, found: 562.195.

2-[(4-{[5-(2-{[(4-Fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}phenyl)formamido]acetic acid (15) was synthesized according to the general procedure of the ester hydrolysis from intermediate **11**. **15** was obtained with 17 % yield over two steps as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.79 (br s, 1H), 9.17 (t, *J* = 6.4 Hz, 1H), 8.43 (s, 1H), 8.05 (br s, 1H), 7.84-7.75 (m, 2H), 7.45-7.35 (m, 2H), 7.14 (dd, *J* = 11.4 Hz, 8.3 Hz, 1H), 7.07 (dd, *J* = 8.3 Hz, 1.6 Hz, 1H), 6.80 (ddd, *J* = 8.0 Hz, 4.3 Hz, 1.9 Hz, 1H), 4.97 (s, 2H), 4.27 (d, *J* = 6.1 Hz, 2H), 3.81 (s, 3H),

3.56 (br s, 2H) ppm. ^{13}C NMR (125 MHz, DMF-d_7, 25 °C, TMS): δ 166.4, 163.7, 158.8, 152.8, 152.3 (d, J_{CF} = 243.5 Hz), 151.6, 148.5 (d, J_{CF} = 10.9 Hz), 140.5, 137.0 (d, J_{CF} = 3.6 Hz), 135.9 , 128.9 (2C), 128.5 (2C), 120.8 (d, J_{CF} = 7.6 Hz), 116.6 (d, J_{CF} = 17.6 Hz), 114.5 (d, J_{CF} = 2.0 Hz), 97.3, 88.1, 78.6, 56.8, 57.3, 44.6, 43.5 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{25}H_{21}FN_4O_7$: 508.139, found: 508.149.

(2*S*)-2-[(4-{[5-(2-{[(4-Fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}phenyl)formamido]propanoic acid (16) was synthesized according to the general procedure of the ester hydrolysis from intermediate **12**. **16** was obtained with 18 % yield over two steps as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 12.54 (br s, 1H), 11.83 (s, 1H), 9.17 (t, *J* = 6.0 Hz, 1H), 8.60 (s, 1H), 8.44 (s, 1H), 7.89-7.82 (m, 2H), 7.47-7.82 (m, 2H), 7.15 (dd, *J* = 11.3 Hz, 8.2 Hz, 1H), 7.08 (dd, *J* = 8.4 Hz, 1.6 Hz, 1H), 6.81 (ddd, *J* = 8.1 Hz, 4.1 Hz, 1.5 Hz, 1H), 4.98 (s, 2H), 4.36 (m, 1H), 4.28 (d, *J* = 6.0 Hz, 2H), 3.82 (s, 3H), 1.38 (m, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 162.1 (2C), 152.7, 152.1, 151.0 (d, J_{CF} = 242.8 Hz), 150.4, 147.3 (d, J_{CF} = 9.6 Hz), 139.8, 135.9 (d, J_{CF} = 3.5 Hz), 134.1, 130.0, 128.2 (2C), 127.1 (2C), 120.0 (d, J_{CF} = 6.9 Hz), 116.0 (d, J_{CF} = 18.2 Hz), 113.6 (d, J_{CF} = 1.3 Hz), 96.0, 87.4, 78.1, 56.4, 51.3, 49.1, 42.5, 17.6 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{26}H_{23}FN_4O_7$: 522.155, found: 522.161.

(2*R*)-2-[(4-{[5-(2-{[(4-Fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}phenyl)formamido]propanoic acid (17) was synthesized according to the general procedure of the ester hydrolysis from intermediate **47**. **17** was obtained with 28 % yield over two steps as a white solid. ^{1}H NMR (500 MHz, MeOD-d_4, 25 °C, TMS): δ 8.30 (s, 1H), 7.93-7.85 (m, 2H), 7.51-7.44 (m, 2H), 7.09-7.02 (m, 2H), 6.87 (ddd, *J* = 7.8 Hz, 4.0 Hz, 1.8 Hz, 1H), 5.06 (s, 2H), 4.46 (q, *J* = 7.1 Hz, 1H), 4.40 (s, 2H), 3.88 (s, 3H),

1.48 (d, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (125 MHz, MeOD-d_4, 25 °C, TMS): δ 167.0, 163.0, 153.8, 151.7 (d, J_{CF} = 244.3 Hz), 151.5, 150.1, 147.7 (d, J_{CF} = 11.6 Hz), 139.0, 134.4, 134.3 (d, J_{CF} = 4.0 Hz), 127.6 (2C), 127.5 (2C), 119.7 (d, J_{CF} = 7.1 Hz), 115.3 (d, J_{CF} = 19.2 Hz), 113.0 (d, J_{CF} = 1.9 Hz), 96.1, 86.5, 77.5, 55.3, 51.3, 48.4, 42.6, 17.9 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{26}H_{23}FN_4O_7$: 522.166, found: 522.163.

(2*S*)-2-[(4-{[5-(2-{[(4-Fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}phenyl)formamido]-3-hydroxypropanoate (18) was synthesized according to the general procedure of the ester hydrolysis from intermediate **13**. **18** was obtained with 23 % yield over two steps as a white solid. ^{1}H NMR (500 MHz, DMF-d_7, 25 °C, TMS): δ 9.23 (t, J = 5.7 Hz, 1H), 8.54 (s, 1H), 8.90 (br s, 1H), 7.98-7.84 (m, 2H), 7.60-7.48 (m, 2H), 7.21 (dd, J = 8.6 Hz, 1.7 Hz, 1H), 7.16 (dd, J = 11.6 Hz, 8.3 Hz, 1H), 6.92 (ddd, J = 7.7 Hz, 3.8 Hz, 1.5 Hz, 1H), 5.12 (s, 2H), 4.42 (d, J = 5.8 Hz, 2H), 4.26-4.15 (m, 1H), 3.89 (s, 3H), 3.71-3.60 (m, 2H) ppm. ^{13}C NMR (125 MHz, DMF-d_7, 25 °C, TMS): δ 166.6, 163.7, 153.8, 152.7, 152.3 (d, J_{CF} = 243.0 Hz), 151.5, 148.5 (d, J_{CF} = 10.9 Hz), 140.6, 137.0 (d, J_{CF} = 3.7 Hz), 135.8, 129.0 (2C), 128.9, 128.5 (2C), 120.8 (d, J_{CF} = 6.8 Hz), 116.6 (d, J_{CF} = 18.0 Hz), 114.5 (d, J_{CF} = 2.0 Hz), 97.3, 88.1, 78.5, 64.2, 56.8, 52.2, 49.9, 43.5 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{26}H_{23}FN_4O_8$: 538.150, found: 538.159.

(2*R*)-2-[(4-{[5-(2-{[(4-Fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}phenyl)formamido]-3-hydroxypropanoic acid (19) was synthesized according to the general procedure of the ester hydrolysis from intermediate **48**. **19** was obtained with 7 % yield over two steps as a white solid. ^{1}H NMR (500 MHz, MeOD-d_4, 25 °C, TMS): δ 8.29 (s, 1H), 7.96-7.90 (m, 2H), 7.52-7.44 (m, 2H), 7.09-7.02 (m, 2H), 7.09-7.02 (m, 2H), 6.87 (ddd, J = 8.3 Hz, 4.2 Hz, 2.1 Hz, 1H), 5.06 (s, 2H), 4.54 (s, 2H), 4.54 (br s, 1H),

4.40 (s, H), 4.00 (br s, 2H), 3.88 (s, 3H) ppm. ^{13}C NMR (125 MHz, MeOD-d_4, 25 °C, TMS): δ 167.6, 163.0, 153.8, 151.5, 151.4 (d, J_{CF} = 245.7 Hz), 150.1, 150.1, 147.7 (d, J_{CF} = 10.9 Hz), 139.1, 135.4 (d, J_{CF} = 3.4 Hz), 134.3, 127.7 (2C), 127.6 (2C), 119.7 (d, J_{CF} = 6.8 Hz), 115.3 (d, J_{CF} = 18.8 Hz), 113.0 (d, J_{CF} = 1.1 Hz), 96.1, 86.6, 77.5, 65.6, 55.3, 51.3, 42.7, 39.0 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{26}H_{23}FN_4O_8$: 538.150, found: 538.159.

(2*R*)-1-(4-{[5-(2-{[(4-Fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}benzoyl)pyrrolidine-2-carboxylic acid (20) was synthesized according to the general procedure of the ester hydrolysis from intermediate **14**. **20** was obtained with 19 % yield over two steps as a slight yellow solid. ^{1}H NMR (500 MHz, MeOD-d_4, 25 °C, TMS): δ (mixture of rotamers) 8.30 (s, 0.6H), 8.26 (s, 0.4H), 7.65-7.61 (m, 1.1H), 7.55-7.38 (m, 2.9H), 7.10-7.01 (m, 2H), 6.90-6.84 (m, 1H), 5.04 (s, 1.2H), 5.01 (s, 0.8H), 4.40 (s, 2H), 3.88 (s, 3H), 3.81-3.59 (m, 2H), 3.55-3.44 (m, 1H), 2.43-1.82 (m, 4H) ppm. ^{13}C NMR (125 MHz, MeOD-d_4, 25 °C, TMS): δ (mixture of rotamers) 170.7, 169.8, 163.0, 163.0, 153.8, 152.7, 151.8, 151.5, 151.5, 150.7, 150.1, 150.1, 147.7, 147.6, 137.7, 137.1, 137.1, 136.5, 134.4, 134.4, 134.3, 127.8, 127.6, 127.5, 127.2, 119.7, 119.7, 115.4, 115.4, 115.3, 115.3, 113.0, 113.0, 113.0, 96.1, 96.0, 86.6, 86.5, 77.5, 55.3, 51.3, 51.2, 50.0, 46.5, 42.6, 39.0, 31.4, 29.5, 24.9, 22.3 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{28}H_{25}FN_4O_7$: 548.171, found: 548.178.

3-{2,4-Dioxo-1-[(4-sulfamoylphenyl)methyl]-1,2,3,4-tetrahydropyrimidin-5-yl}-*N*-[(4-fluoro-3-methoxyphenyl)methyl]prop-2-ynamide (21) was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **55** and **31**. Before flash chromatography, the product was washed with 10 ml of a 1/1 mixture of methanol/water. **21** was obtained with 36 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.84 (s, 1H), 9.17 (t, *J* = 6.1 Hz, 1H), 8.43 (s, 1H), 7.84-7.76 (m, 2H), 7.55-7.48 (m, 2H), 7.36 (s, 2H),

7.15 (dd, J = 11.5 Hz, 8.3 Hz, 1H), 7.08 (dd, J = 8.4 Hz, 2.1 Hz, 1H), 6.81 (ddd, J = 8.2 Hz, 4.4 Hz, 2.1 Hz, 1H), 5.00 (s, 2H), 4.28 (d, J = 6.1 Hz, 2H), 3.82 (s, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 157.5, 152.7, 151.6 (d, J_{CF} = 241.9 Hz), 152.0, 150.1, 147.3 (d, J_{CF} = 10.4 Hz), 143.9, 140.7, 135.9 (d, J_{CF} = 3.6 Hz), 128.4 (2C), 126.4 (2C), 120.0 (d, J_{CF} = 7.0 Hz), 116.0 (d, J_{CF} = 18.1 Hz), 113.6, 96.1, 87.3, 78.4, 56.4, 51.3, 42.5 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{22}H_{19}FN_4O_6S$: 486.101, found: 486.108.

***N*-[(4-Fluoro-3-methoxyphenyl)methyl]-3-(1-{[4-(methylsulfamoyl)phenyl]methyl}-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)prop-2-ynamide (22)** was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **56** and **31**. **22** was obtained with 42 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.70 (s, 1H), 9.18 (t, J = 6.1 Hz, 1H), 8.48 (s, 1H), 7.79-7.74 (m, 2H), 7.57-7.51 (m, 2H), 7.47 (q, J = 5.0 Hz, 1H), 7.15 (dd, J = 11.6 Hz, 8.3 Hz, 1H), 7.08 (dd, J = 8.4 Hz, 2.1 Hz, 1H), 6.82 (ddd, J = 8.2 Hz, 4.3 Hz, 2.0 Hz, 1H), 5.02 (s, 2H), 4.29 (d, J = 6.1 Hz, 2H), 3.82 (s, 3H), 2.41 (d, J = 4.9 Hz, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 162.1, 152.7, 152.2, 151.1 (d, J_{CF} = 242.6 Hz), 150.4, 147.3 (d, J_{CF} = 10.8 Hz), 141.2, 139.1, 135.9 (d, J_{CF} = 3.5 Hz), 128.6 (2C), 127.5 (2C), 120.0 (d, J_{CF} = 6.9 Hz), 116.0 (d, J_{CF} = 18.1 Hz), 113.6, 96.2, 87.4, 78.1, 56.4, 51.3, 42.5, 29.1 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{23}H_{21}FN_4O_6S$: 500.117, found: 500.125.

3-(1-{[4-(Dimethylsulfamoyl)phenyl]methyl}-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-*N*-[(4-fluoro-3-methoxyphenyl)methyl]prop-2-ynamide (23) was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **57** and **31**. **23** was obtained with 15 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.85 (s, 1H), 9.17 (t, J = 6.1 Hz, 1H), 8.47 (s, 1H), 7.77-7.72 (m, 2H), 7.60-7.56 (m, 2H), 7.15 (dd, J = 11.5

Hz, 8.2 Hz, 1H), 7.08 (dd, *J* = 8.4 Hz, 2.1 Hz, 1H), 6.82 (ddd, *J* = 8.1 Hz, 4.2 Hz, 2.0 Hz, 1H), 5.03 (s, 2H), 4.28 (d, *J* = 6.1 Hz, 2H), 3.82 (s, 3H), 2.61 (s, 6H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 162.6, 152.7, 152.1, 151.1 (d, J_{CF} = 242.4 Hz), 150.7, 147.3 (d, J_{CF} = 10.5 Hz), 142.1, 136.0 (d, J_{CF} = 3.6 Hz), 134.6, 128.7 (2C), 128.4 (2C), 120.0 (d, J_{CF} = 6.7 Hz), 116.0 (d, J_{CF} = 18.1 Hz), 113.6 (d, J_{CF} = 1.3 Hz), 96.2, 87.3, 78.4, 56.4, 51.2, 42.5, 38.0 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{24}H_{23}FN_4O_6S$: 514.132, found: 514.141.

3-[2,4-Dioxo-1-({4-[(propan-2-yl)sulfamoyl]phenyl}methyl)-1,2,3,4-tetrahydropyrimidin-5-yl]-*N*-[(4-fluoro-3-methoxyphenyl)methyl]prop-2-ynamide (24) was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **58** and **31**. **24** was obtained with 44 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.81 (s, 1H), 9.16 (t, *J* = 6.1 Hz, 1H), 8.44 (s, 1H), 7.81-7.77 (m, 2H), 7.60 (d, *J* = 7.1 Hz, 1H), 7.54-7.48 (m, 2H), 7.15 (dd, *J* = 11.6 Hz, 8.3 Hz, 1H), 7.08 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H), 6.81 (ddd, *J* = 8.3 Hz, 4.4 Hz, 2.1 Hz, 1H), 5.01 (s, 2H), 4.28 (d, *J* = 6.1 Hz, 2H), 3.82 (s, 3H), 3.22 (octet, *J* = 6.7 Hz, 1H), 0.94 (d, *J* = 6.5 Hz, 6H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 162.5, 152.7, 152.1, 151.1 (d, J_{CF} = 243.2 Hz), 150.7, 147.3 (d, J_{CF} = 10.6 Hz), 141.6, 141.1, 135.9 (d, J_{CF} = 3.4 Hz), 128.4 (2C), 127.2 (2C), 120.0 (d, J_{CF} = 7.0 Hz), 116.0 (d, J_{CF} = 18.1 Hz), 113.6 (d, J_{CF} = 1.3 Hz), 96.1, 87.3, 78.4, 56.4, 51.3, 47.7, 42.5, 23.7 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{25}H_{25}FN_4O_6S$: 528.148, found: 528.155.

3-(1-{[4-(Cyclopropylsulfamoyl)phenyl]methyl}-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-*N*-[(4-fluoro-3-methoxyphenyl)methyl]prop-2-ynamide (25) was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **59** and **31**. **25** was obtained with 48 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 9.16 (t, *J* = 6.1 Hz, 1H), 8.43 (s, 1H), 7.94 (s, 1H), 7.82-7.77 (m, 2H), 7.56-7.51 (m, 2H), 7.15 (dd, *J* =

11.6 Hz, 8.3 Hz, 1H), 7.08 (dd, *J* = 8.4 Hz, 2.1 Hz, 1H), 6.81 (ddd, *J* = 8.2 Hz, 4.4 Hz, 2.1 Hz, 1H), 5.01 (s, 2H), 4.28 (d, *J* = 6.1 Hz, 2H), 3.82 (s, 3H), 2.10-2.04 (m, 1H), 0.50-0.45 (m, 2H), 0.41-0.35 (m, 1H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 162.9, 152.8, 151.1 (d, J_{CF} = 242.9 Hz), 152.0, 150.9, 147.3 (d, J_{CF} = 10.9 Hz), 141.5, 140.0, 136.0 (d, J_{CF} = 3.6 Hz), 128.5 (2C), 127.6 (2C), 120.0 (d, J_{CF} = 6.7 Hz), 116.0 (d, J_{CF} = 18.1 Hz), 113.6, 96.1, 87.3, 78.6, 56.4, 51.3, 42.5, 24.6, 5.6 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{25}H_{23}FN_4O_6S$: 526.132, found: 526.138.

3-(1-{[4-(tert-Butylsulfamoyl)phenyl]methyl}-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-*N*-[(4-fluoro-3-methoxyphenyl)methyl]prop-2-ynamide (26) was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **60** and **31**. **26** was obtained with 53 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.86 (s, 1H), 9.16 (t, *J* = 6.1 Hz, 1H), 8.44 (s, 1H), 7.83-7.78 (m, 2H), 7.55 (s, 1H), 7.51-7.46 (m, 2H), 7.15 (dd, *J* = 11.5 Hz, 8.3 Hz, 1H), 7.08 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H), 6.81 (ddd, *J* = 8.2 Hz, 4.4 Hz, 2.1 Hz, 1H), 5.00 (s, 2H), 4.28 (d, *J* = 6.1 Hz, 2H), 3.82 (s, 3H), 1.09 (s, 9H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 162.5, 152.7, 152.1, 151.1 (d, J_{CF} = 242.3 Hz), 150.6, 147.3 (d, J_{CF} = 10.4 Hz), 144.1, 140.7, 136.0 (d, J_{CF} = 3.5 Hz), 128.2 (2C), 127.0 (2C), 120.0 (d, J_{CF} = 7.0 Hz), 116.0 (d, J_{CF} = 18.1 Hz), 113.6, 96.1, 87.3, 78.4, 56.4, 53.8, 51.3, 42.5, 30.2 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{26}H_{27}FN_4O_6S$: 542.164, found: 542.173.

***N*-[(4-Fluoro-3-methoxyphenyl)methyl]-3-(1-{[4-(morpholine-4-sulfonyl)phenyl]methyl}-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)prop-2-ynamide (27)** was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **61** and **31**. **27** was obtained with 27 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.87 (s, 1H), 9.18 (t, *J* = 6.1 Hz, 1H), 8.49 (s, 1H), 7.76-7.72 (m, 2H), 7.62-7.58 (m, 2H), 7.15 (dd, *J* =

11.6 Hz, 8.3 Hz, 1H), 7.08 (dd, *J* = 8.4 Hz, 2.1 Hz, 1H), 6.82 (ddd, *J* = 8.2 Hz, 4.3 Hz, 2.1 Hz, 1H), 5.05 (s, 2H), 4.29 (d, *J* = 6.1 Hz, 2H), 3.82 (s, 3H), 3.65-3.60 (m, 4H), 2.88-2.83 (m, 4H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 162.2, 152.7, 152.1, 151.1 (d, J_{CF} =242.7 Hz), 150.5, 147.3 (d, J_{CF} = 10.8 Hz), 142.4, 135.9 (d, J_{CF} = 3.6 Hz), 134.2, 128.7 (2C), 128.5 (2C), 120.0 (d, J_{CF} = 7.0 Hz), 116.0 (d, J_{CF} = 18.0 Hz), 113.6 (d, J_{CF} = 1.2 Hz), 96.2, 87.4, 78.1, 65.7 (2C), 56.4, 51.3, 46.3 (2C), 42.5 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{26}H_{25}FN_4O_7S$: 556.143, found: 556.152.

Methyl 2-(4-{[5-(2-{[(4-fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}benzenesulfonamido)acetate (28) was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **62** and **31**. **28** was obtained with 9 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.79 (s, 1H), 9.16 (t, *J* = 6.1 Hz, 1H), 8.43 (s, 1H), 8.23 (s, 2H), 7.80-7.74 (m, 2H), 7.54-7.48 (m, 2H), 7.15 (dd, *J* = 11.5 Hz, 8.3 Hz, 1H), 7.08 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H), 6.81 (ddd, *J* = 8.2 Hz, 4.3 Hz, 2.1 Hz, 1H), 5.00 (s, 2H), 4.28 (d, *J* = 6.1 Hz, 2H), 3.82 (s, 3H), 3.70 (s, 2H), 3.50 (s, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 169.8, 162.5, 152.7, 152.0, 151.1 (d, J_{CF} = 242.7 Hz), 150.7, 147.3 (d, J_{CF} = 10.5 Hz), 141.4, 140.4, 136.0 (d, J_{CF} = 3.6 Hz), 128.5 (2C), 127.3 (2C), 120.0 (d, J_{CF} = 7.1 Hz), 116.0 (d, J_{CF} = 18.1 Hz), 113.6, 96.1, 87.3, 78.4, 56.4, 52.2, 51.2, 44.2, 42.5 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{25}H_{23}FN_4O_8S$: 558.122, found: 558.129.

5-(4-Methylphenyl)-1,3-oxazole (29). 4-Methylbenzaldehyde (10.2 mmol, 1.0 eq), potassium carbonate (11.1 mmol, 1.1 eq) and TOSMIC (10.5 mmol, 1.0 eq) were dissolved in methanol (36 mL) and stirred in a closed vessel for 2 h at 80 °C in the microwave. The reaction mixture was diluted with water and the methanol was evaporated under reduced pressure. The aqueous layer

was then extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtrated and concentrated in vacuo. The crude product was purified by flash chromatography (SiO_2, gradient: 100 % cyclohexane – 100 % ethyl acetate) affording **29** as a slight yellow solid (774 mg, yield = 48 %). ^{1}H NMR (500 MHz, $CDCl_3$-*d*, 25 °C, TMS): δ 7.91 (s, 1H), 7.61-7.54 (m, 2H), 7.32 (s, 1H), 7.28-7.23 (m, 2H), 2.41 (s, 3H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$-*d*, 25 °C, TMS): δ 151.7, 150.1, 138.7, 129.6 (2C), 125.1, 124.4 (2C), 120.8, 21.4 ppm.

5-Iodo-1-{[4-(1,3-oxazol-5-yl)phenyl]methyl}-1,2,3,4-tetrahydropyrimidine-2,4-dione (30) was synthesized over two steps without purification of the intermediate. **29** (4.7 mmol, 1.0 eq), *N*-bromosuccinimide (NBS, 4.9 mmol, 1.0 eq) and azobis(isobutyronitrile) (AIBN, 0.5 mmol, 0.1 eq) were dissolved in tetrachloromethane (55 mL), and stirred over night at 80 °C. After cooling to room temperature, the reaction mixture was diluted with water. The aqueous layer was washed with DCM and the combined organic layers were dried over sodium sulfate, filtrated and concentrated in vacuo, affording an intensively yellow solid. The solid was then dissolved in DMF (7 mL) and added dropwise to a suspension of 5-iodouracil (8.4 mmol, 1.8 eq) and caesium carbonate (5.7 mmol, 1.2 eq) in DMF (18 mL), which has been stirred at room temperature for 15 minutes before under nitrogen atmosphere. After the complete addition, the mixture was stirred for 2.5 h. The mixture was then separated between ethyl acetate and hydrochloric acid (2N). The combined organic layers were then concentrated in vacuo, resulting in a solution in DMF, which was purified by flash chromatography (reversed phase C18, gradient: 10 % methanol in water – 100 % methanol) affording **30** as a slightly yellow solid (400 mg, yield = 22 %). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.73 (s, 1H), 8.45 (s, 1H), 8.38 (s, 1H), 7.75-7.71 (m, 2H), 7.69 (s, 1H), 7.45-7.41 (m, 2H), 4.91 (s, 2H) ppm. ^{13}C NMR (125 MHz,

DMSO-d_6, 25 °C, TMS): δ 161.5, 152.4, 151.2, 150.7, 150.2, 137.6, 128.8 (2C), 127.3, 124.8 (2C), 122.6, 69.3, 50.8 ppm.

***N*-[(4-Fluoro-3-methoxyphenyl)methyl]prop-2-ynamide (31)** was synthesized over two steps, without purification after the first step. Lithium aluminum hydride (36.6 mmol) was suspended in THF (50 mL) and cooled to 0 °C under nitrogen atmosphere. 4-fluoro-3-methoxybenzonitrile (13.4 mmol) was added in one portion and the mixture was stirred for one hour at 0 °C. After warming to room temperature, the reaction mixture was stirred over night. The reaction was stopped by adding water (1.5 mL), sodium hydroxide solution (15 %, 1.5 mL) and water (4.75 mL) at 0 °C. The suspension was stirred for 30 minutes at 0 °C and then filtrated. The filtrate was concentrated in vacuo. The resulting yellow liquid was dissolved in dichloromethane (DCM, 70 mL) and EEDQ (4.81 g, 19.5 mmol) was added at room temperature and under nitrogen atmosphere. To this solution propiolic acid (1.40g, 19.7 mmol) was added dropwise. The yellow reaction mixture was stirred over night and then separated between ethyl acetate and hydrochloric acid (1N). After drying over sodium sulfate the organic layers were concentrated in vacuo. The crude product was purified by flash chromatography (reversed phase C18, gradient: 10 % methanol in water – 100 % methanol) affording **31** as a brown solid (2.2 g, yield = 79 %). ^{1}H NMR (500 MHz, $CDCl_3$-*d*, 25 °C, TMS): δ 7.05 (dd, J = 11.1 Hz, 8.2 Hz, 1H), 6.92 (dd, J = 8.0 Hz, 2.0 Hz, 1H), 6.82 (ddd, J = 8.2 Hz, 4.2 Hz, 2.2 Hz, 1H), 6.26 (br s, 1H), 4.45 (d, J = 6.0 Hz, 2H), 3.90 (s, 3H), 2.84 (s, 1H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$-*d*, 25 °C, TMS): δ 152.0 (d, J_{CF} = 245.7 Hz), 152.0, 147.9 (d, J_{CF} = 11.2 Hz), 133.4 (d, J_{CF} = 3.3 Hz), 120.2 (d, J_{CF} = 6.3 Hz), 116.2 (d, J_{CF} = 19.5 Hz), 113.3 (d, J_{CF} = 2.0 Hz), 77.2, 73.8, 56.3, 43.5 ppm.

4-[(5-Iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]benzoic acid (32) was synthesized according to the general procedure for the substitution by 5-iodouracil applied on 4-

(bromomethyl)benzoic acid. **32** was obtained with 17 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 12.99 (br s, 1H), 11.74 (s, 1H), 8.37 (s, 1H), 7.96-7.89 (m, 2H), 7.44-7.37 (m, 2H), 4.95 (s, 2H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 167.5, 161.5, 151.2, 150.3, 140.0, 130.7, 130.1 (2C), 127.9 (2C), 69.4, 50.9 ppm.

Methyl 4-[(5-iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]benzoate (33) was synthesized according to the general procedure for the substitution by 5-iodouracil applied on methyl 4-(bromomethyl)benzoate. **33** was obtained with 31 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.74 (s, 1H), 8.37 (s, 1H), 7.99-7.92 (m, 2H), 7.48-7.41 (m, 2H), 4.96 (s, 2H), 3.85 (s, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 166.4, 161.5, 151.2, 150.3, 142.6, 1300 (2C), 129.4, 128.0 (2C), 69.4, 52.6, 50.9 ppm.

4-[(5-Iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]benzamide (34) was synthesized according to the general procedure for the amide formation procedure A from intermediate **32** and ammonium chloride. **34** was obtained with 67 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.73 (s, 1H), 8.36 (s, 1H), 7.96 (br s, 1H), 7.87-7.81 (m, 2H), 7.41-7.30 (m, 3H), 4.93 (s, 2H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 168.0, 161.5, 151.2, 150.2, 140.3, 143.2, 127.3 (2C), 127.7 (2C), 69.3, 50.8 ppm.

4-[(5-Iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]-*N*-methylbenzamide (35) was synthesized according to the general procedure for the amide formation procedure A from intermediate **32** and methyl amine. **35** was obtained with 78 % yield as a slight yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.73 (s, 1H), 8.42 (q, J = 4.6 Hz, 1H), 8.36 (s, 1H), 7.83-7.77 (m, 2H), 7.41-7.33 (m, 2H), 4.92 (s, 2H), 2.78 (d, J = 4.5 Hz, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 166.7, 161.5, 151.2, 150.2, 140.0, 134.4, 127.8 (2C), 127.7 (2C), 69.3, 50.8, 26.7 ppm.

4-[(5-Iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]-*N,N*-dimethylbenzamide (36) was synthesized according to the general procedure for the amide formation procedure A from intermediate **32** and dimethyl amine. **36** was obtained with 56 % yield as a slight yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.73 (s, 1H), 8.38 (s, 1H), 7.42-7.38 (m, 2H), 7.38-7.31 (m, 2H), 4.91 (s, 2H), 2.98 (br s, 3H), 2.90 (br s, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 170.2, 161.5, 151.2, 150.2, 138.3, 136.3, 127.8 (2C), 127.7 (2C), 69.3, 50.8, 39.4, 35.2 ppm.

4-[(5-Iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]-*N*-(propan-2-yl)benzamide (37) was synthesized according to the general procedure for the amide formation procedure A from intermediate **32** and isopropyl amine. **37** was obtained with 55 % yield as a white solid. ^{1}H NMR (500 MHz, MeOD-d_4, 25 °C, TMS): δ 8.23 (d, J = 7.5 Hz, 1H), 8.18 (s, 1H), 7.85-7.79 (m, 2H), 7.46-7.40 (m, 2H), 5.01 (s, 2H), 4.21 (octet, J = 7.2 Hz, 1H), 1.26 (d, J = 6.7 Hz, 6H) ppm. ^{13}C NMR (125 MHz, MeOD-d_4, 25 °C, TMS): δ 167.7, 161.8, 151.3, 150.0, 139.4, 134.6, 127.5 (2C), 127.4 (2C), 67.4, 50.7, 41.9, 12.2 (2C) ppm.

***N*-Cyclopropyl-4-[(5-iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]benzamide (38)** was synthesized according to the general procedure for the amide formation procedure A from intermediate **32** and cyclopropyl amine. **38** was obtained with 49 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.73 (s, 1H), 8.41 (d, J = 4.2 Hz, 1H), 8.33 (s, 1H), 7.82-7.74 (m, 2H), 7.40-7.32 (m, 2H), 4.91 (s, 2H), 2.86-2.80 (m, 1H), 0.72-0.65 (m, 2H), 0.59-0.51 (m, 2H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 169.8, 163.8, 153.5, 152.4, 142.3, 136.5, 130.2 (2C), 129.9 (2C), 71.6, 53.0, 25.7, 8.4 (2C) ppm.

***N*-tert-Butyl-4-[(5-iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]benzamide (39)** was synthesized according to the general procedure for the amide formation procedure A from

intermediate **32** and tert-butyl amine. **39** was obtained with 17 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.72 (s, 1H), 8.35 (s, H), 7.78-7.74 (m, 2H), 7.72 (s, 1H), 7.39-7.33 (m, 2H), 4.92 (s, 2H), 1.37 (s, 9H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 166.5, 161.5, 151.2, 150.2, 139.7, 153.9, 128.1 (2C), 127.6 (2C), 69.3, 51.2, 50.7, 29.0 (3C) ppm.

***N*-(2-Hydroxyethyl)-4-[(5-iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]benzamide (40)** was synthesized according to the general procedure for the amide formation procedure A from intermediate **32** and ethanolamine, but was crystallized in methanol/water instead of chromatography. **40** was obtained with 48 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.58 (s, 1H), 8.42 (t, J = 5.2 Hz, 1H), 8.34 (s, 1H), 7.86-7.80 (m, 2H), 7.41-7.34 (m, 2H), 4.92 (s, 2H), 4.72 (t, J = 5.1 Hz, 1H), 3.50 (q, J = 5.9 Hz, 2H), 3.33 (q, J = ca. 5.9 Hz, 2H)[1] ppm.

[1] Signal covered by water signal. Only 1H NMR spectra were determined because of analogy to all other compounds.

Methyl 2-({4-[(5-iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]phenyl}formamido)acetate (41) was synthesized according to the general procedure for the amide formation procedure A from intermediate **32** and glycine methyl ester hydrochloride. **41** was obtained with 69 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.73 (s, 1H), 8.95 (t, J = 6.1 Hz, 1H), 8.37 (s, 1H), 7.87-7.83 (m, 2H), 7.42-7.39 (m, 2H), 4.94 (s, 2H), 4.02 (d, J = 6.0 Hz, 2H), 3.66 (s, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 170.8, 161.5, 151.2, 150.2, 140.6, 133.4, 128.1 (2C), 127.8 (2C), 69.4, 52.2, 50.8, 41.7 ppm.

Methyl (2*S*)-2-({4-[(5-iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]phenyl}formamido)propanoate (42) was synthesized according to the general procedure for the amide formation procedure A from intermediate **32** and L-alanine methyl ester hydrochloride. **42** was obtained with 65 % yield as a white solid. ^{1}H NMR (500 MHz, MeOD-d_4, 25 °C, TMS): δ 8.18 (s, 1H), 7.87-7.83 (m, 2H), 7.45-7.45 (m, 2H), 4.99 (s, 2H), 4.60 (q, J = 7.4 Hz, 1H), 3.73 (s, 3H), 1.49 (d, J = 7.4 Hz, 3H) ppm. ^{13}C NMR (125 MHz, MeOD-d_4, 25 °C, TMS): δ 173.4, 168.2, 161.8, 151.3, 150.0, 139.9, 133.5, 127.7 (2C), 127.5 (2C), 67.1, 51.4, 50.7, 48.7, 15.8 ppm.

Methyl (2*R*)-2-({4-[(5-iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]phenyl}formamido)propanoate (43) was synthesized according to the general procedure for the amide formation procedure A from intermediate **32** and D-alanine methyl ester hydrochloride. **43** was obtained with 49 % yield as a white solid. ^{1}H NMR (500 MHz, MeOD-d_4, 25 °C, TMS): δ 8.19 (s, 1H), 7.89-7.84 (m, 2H), 7.47-7.42 (m, 2H), 5.01 (s, 2H), 4.62 (q, J = 7.4 Hz, 1H), 3.76 (s, 3H), 1.51 (d, J = 7.4 Hz, 3H) ppm. ^{13}C NMR (125 MHz, MeOD-d_4, 25 °C, TMS): δ 173.4, 168.2, 161.8, 151.3, 150.0, 139.9, 133.5, 127.7 (2C), 127.4 (2C), 67.1, 51.4, 50.7, 48.7, 15.8 ppm.

Methyl (2*S*)-3-hydroxy-2-({4-[(5-iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]phenyl}formamido)propanoate (44) was synthesized according to the general procedure for the amide formation procedure A from intermediate **32** and L-serine methyl ester hydrochloride. **44** was obtained with 65 % yield as a white solid. ^{1}H NMR (500 MHz, MeOD-d_4, 25 °C, TMS): δ 8.19 (s, 1H), 7.92-7.87 (m, 2H), 7.48-7.44 (m, 2H), 5.02 (s, 2H), 7.47 (dd, J = 5.2 Hz, 4.3 Hz, 1H), 4.02 (dd, J = 11.4 Hz, 5.3 Hz, 1H), 3.96 (dd, J = 11.4 Hz, 4.2 Hz, 1H), 3.79

(s, 3H) ppm. ^{13}C NMR (125 MHz, MeOD-d_4, 25 °C, TMS): δ 170.9, 168.3, 161.8, 151.3, 150.0, 140.0, 133.5, 127.7 (2C), 127.5 (2C), 67.1, 61.4, 55.5, 51.5, 50.7 ppm.

Methyl (2*R*)-3-hydroxy-2-({4-[(5-iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]phenyl}formamido)propanoate (45) was synthesized according to the general procedure for the amide formation procedure A from intermediate **32** and D-serine methyl ester hydrochloride. **45** was obtained with 118 % yield as a yellow solid. ^{1}H NMR (500 MHz, MeOD-d_4, 25 °C, TMS): δ 8.18 (s, 1H), 7.91-7.86 (m, 2H), 7.47-7.42 (m, 2H), 5.02 (s, 2H), 4.75 (dd, J = 5.1 Hz, 4.2 Hz, 1H), 4.02 (dd, J = 11.5 Hz, 5.3 Hz, 1H), 3.96 (dd, J = 11.4 Hz, 4.2 Hz, 1H), 3.78 (s, 3H) ppm. ^{13}C NMR (125 MHz, MeOD-d_4, 25 °C, TMS): δ 171.0, 168.3, 161.9, 151.3, 150.0, 140.0, 133.4, 127.7 (2C), 127.5 (2C), 67.0, 61.4, 55.5, 51.6, 50.8 ppm.

Methyl (2*R*)-1-{4-[(5-iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]benzoyl}pyrrolidine-2-carboxylate (46) was synthesized according to the general procedure for the amide formation procedure A from intermediate **32** and D-proline methyl ester hydrochloride. **46** was obtained with 74 % yield as a yellow solid. ^{1}H NMR (500 MHz, MeOD-d_4, 25 °C, TMS): δ 8.21 (s, 1H), 7.62-7.57 (m, 2H), 7.48-7.43 (m, 2H), 5.01 (s, 2H), 4.61 (dd, J = 8.4 Hz, 5.3 Hz, 1H), 3.77 (s, 3H), 3.65-3.55 (m, 2H), 2.42-2.34 (m, 1H), 2.05-1.99 (m, 2H), 1.97-1.90 (m, 1H) ppm. ^{13}C NMR (125 MHz, MeOD-d4, 25 °C, TMS): δ 172.9, 170.0, 161.8, 151.3, 150.0, 138.7, 135.5, 127.6 (2C), 127.5 (2C), 67.2, 59.5, 51.5, 50.8, 50.0, 29.1, 24.9 ppm.

Methyl (2*R*)-2-[(4-{[5-(2-{[(4-fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}phenyl)formamido]propanoate (47) was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **43** and **31**. Before flash chromatography, the product was washed with 10 ml of a 1/1 mixture of methanol/water. **47** was obtained with 16 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-

d$_6$, 25 °C, TMS): δ 11.83 (s, 1H), 9.16 (t, *J* = 6.5 Hz, 1H), 8.79 (d, *J* = 6.9 Hz, 1H), 8.42 (s, 1H), 7.90-7.83 (m, 2H), 7.46-7.39 (m, 2H), 7.15 (dd, *J* = 11.8 Hz, 8.4 Hz, 1H), 7.08 (dd, *J* = 8.4 Hz, 1.7 Hz, 1H), 6.81 (ddd, *J* = 8.2 Hz, 4.2 Hz, 2.1 Hz, 1H), 4.98 (s, 2H), 4.48 (quint, *J* = 7.2 Hz, 1H), 4.28 (d, *J* = 6.1 Hz, 2H), 3.82 (s, 3H), 3.64 (s, 3H), 1.40 (d, *J* = 7.3 Hz, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-*d*$_6$, 25 °C, TMS): δ 173.6 (2C), 166.3, 152.7, 152.0, 152.0, 151.0 (d, J_{CF} = 242.5 Hz), 147.3 (d, J_{CF} = 10.7 Hz), 140.2, 135.9 (d, J_{CF} = 3.6 Hz), 133.6, 128.3 (2C), 127.9 (2C), 120.0 (d, J_{CF} = 6.9 Hz), 116.0 (d, J_{CF} = 18.0 Hz), 113.6 (d, J_{CF} = 1.2 Hz), 96.0, 87.3, 56.4, 52.4, 51.3, 48.7, 42.5, 17.2 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{27}H_{25}FN_4O_7$: 536.171, found: 536.180.

Methyl (2*R*)-2-[(4-{[5-(2-{[(4-fluoro-3-methoxyphenyl)methyl]carbamoyl}eth-1-yn-1-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]methyl}phenyl)formamido]-3-hydroxypropanoate (48) was synthesized according to the general procedure of the *Sonogashira* coupling from intermediates **45** and **31**. Before flash chromatography, the product was washed with 10 ml of a 1/1 mixture of methanol/water. **48** was obtained with 26 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 25 °C, TMS): δ 11.83 (s, 1H), 9.17 (t, *J* = 6.1 Hz, 1H), 8.57 (d, *J* = 7.5 Hz, 1H), 8.45 (s, 1H), 7.90-7.85 (m, 2H), 7.47-7.41 (m, 2H), 7.15 (dd, *J* = 11.4 Hz, 8.2 Hz, 1H), 7.08 (dd, *J* = 8.5 Hz, 1.9 Hz, 1H), 6.81 (ddd, *J* = 8.3 Hz, 4.4 Hz, 1.9 Hz, 1H), 5.05 (t, *J* = 6.1 Hz, 1H), 4.99 (s, 2H), 4.56-4.51 (m, 1H), 4.28 (d, *J* = 6.1 Hz, 2H), 3.82 (s, 3H), 3.81-3.77 (m, 2H), 3.56 (s, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-*d*$_6$, 25 °C, TMS): δ 171.5, 166.6, 162.1, 152.7, 152.1, 151.1 (d, J_{CF} = 243.1 Hz), 150.4, 147.3 (d, J_{CF} = 10.4 Hz), 140.1, 135.9 (d, J_{CF} = 3.9 Hz), 133.7, 128.3 (2C), 127.9 (2C), 121.0 (d, J_{CF} = 6.4 Hz), 116.0 (d, J_{CF} = 18.2 Hz), 113.6, 96.0, 87.4, 78.1, 61.5, 56.4, 56.1, 52.4, 51.4, 42.5 ppm. HRMS-TOF *m/z* $[M+H]^+$ calculated for $C_{27}H_{25}FN_4O_8$: 552.166, found: 552.174.

4-(Bromomethyl)benzene-1-sulfonamide (49) was synthesized according to the general procedure for the amide formation procedure B in THF at 0 °C with a 25 % aqueous solution of 4-(bromomethyl)benzenesulfonyl chloride and ammonium chloride. **49** was obtained with 89 % yield as a white solid. The product was not purified and characterized; characterization and purification were done in the next step (**55**).

4-(Bromomethyl)-*N*-(propan-2-yl)benzene-1-sulfonamide (50) was synthesized according to the general procedure for the amide formation procedure B from 4-(bromomethyl)benzenesulfonyl chloride and isopropyl amine in THF at -78 °C. **50** was obtained with 49 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 7.81-7.77 (m, 2H), 7.67-7.64 (m, 2H), 7.62 (d, J = 7.2 Hz, 1H), 4.77 (s, 2H), 3.25 (octet, J = 7.0 Hz, 1H), 0.95 (d, J = 6.5 Hz, 6H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 142.7, 142.0, 130.4 (2C), 127.2 (2C), 45.7, 33.3, 23.7 ppm.

4-(Bromomethyl)-*N*-cyclopropylbenzene-1-sulfonamide (51) was synthesized according to the general procedure for the amide formation procedure B from 4-(bromomethyl)benzenesulfonyl chloride and cyclopropyl amine in THF at -78 °C. **51** was obtained with 48 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 7.95 (d, J = 2.7 Hz, 1H), 7.83-7.77 (m, 2H), 7.70-7.66 (m, 2H), 4.78 (s, 1H), 2.21-2.05 (m, 1H), 0.52-0.46 (m, 2H), 0.40-0.35 (m, 2H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 143.1, 140.4, 130.4 (2C), 127.7 (2C), 33.3, 24.6, 5.6 (2C) ppm.

Methyl 2-[4-(bromomethyl)benzenesulfonamido]acetate (52) was synthesized according to the general procedure for the amide formation procedure B from 4-(bromomethyl)benzenesulfonyl chloride and glycine methyl ester hydrochloride in methanol at -55 °C and warmed to -9 °C after 30 min. **52** was obtained with 52 % yield as a white solid. ^{1}H

NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 8.27 (t, J = 6.2 Hz, 1H), 7.81-7.77 (m, 2H), 7.66-7.62 (m, 2H), 4.85 (s, 2H), 3.73 (d, J = 6.2 Hz, 2H), 3.51 (s, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 169.8, 142.5, 140.9, 129.9 (2C), 127.3 (2C), 52.3, 45.4, 33.3 ppm.

***N*-4-Dimethylbenzene-1-sulfonamide (53)** was synthesized according to the general procedure for the amide formation procedure B in THF at room temperature with a 40 % aqueous solution of methylamine and p-toluenesulfonyl chloride. **53** was obtained with 97 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 7.70-7.64 (m, 2H), 7.43-7.38 (m, 2H), 7.35 (br s, 1H), 2.39 (s, 6H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 143.1, 136.9, 130.1 (2C), 127.2 (2C), 29.1, 21.4 ppm.

***N*-tert-Butyl-4-methylbenzene-1-sulfonamide (54)** was synthesized according to the general procedure for the amide formation procedure B in THF at room temperature with tert-butyl amine and p-toluenesulfonyl chloride. **54** was obtained with 98 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 7.75-7.68 (m, 2H), 7.43 (s, 1H), 7.39-7.32 (m, 2H), 2.37 (s, 3H), 1.08 (s, 9H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 142.4, 142.0, 129.8 (2C), 126.8 (2C), 53.6, 30.2, 21.4 (3C) ppm.

4-[(5-Iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]benzene-1-sulfonamide (55) was synthesized according to the general procedure for the substitution by 5-iodouracil applied on intermediate **49**. **55** was obtained with 62 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.73 (s, 1H), 8.35 (s, 1H), 7.84-1.77 (m, 2H), 7.53-7.44 (m, 2H), 7.35 (s, 2H), 4.95 (s, 2H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 161.9, 151.5, 150.1, 143.8, 141.1, 128.3 (2C), 126.4 (2C), 69.7, 50.8 ppm.

4-[(5-Iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]-*N*-methylbenzene-1-sulfonamide (56) was synthesized over two steps without purification of the intermediate. **53**

(2.70 mmol, 1.0 eq), NBS (2.97 mmol, 1.1 eq) and DBPO (dibenzoyl peroxide, 75 %, 0.27 mmol, 0.1 eq) were dissolved in tetrachloromethane (15 mL) and stirred over night at 80 °C. After cooling to room temperature, the reaction mixture was concentrated in vacuo and then partly purified by flash chromatography (SiO_2, gradient: cyclohexane – 100 % ethyl acetate). The intermediate was then substituted with 5-iodouracil as described in the general procedure. **56** was obtained with 48 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.75 (s, 1H), 8.39 (s, 1H), 7.80-7.74 (m, 2H), 7.55-7.49 (m, 2H), 7.46 (q, J = 5.0 Hz, 1H), 4.97 (s, 2H), 2.41 (d, J = 5.0 Hz, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 161.6, 151.2, 150.2, 141.7, 139.0, 128.4 (2C), 127.5 (2C), 69.5, 50.8, 29.1 ppm.

4-[(5-Iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]-*N*,*N*-dimethylbenzene-1-sulfonamide (57) was synthesized in analogy to **56** from commercially available 4-(bromomethyl)-*N*,*N*-dimethylbenzenesulfonamide. **57** was obtained with 33 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.76 (s, 1H), 8.41 (s, 1H), 7.77-7.73 (m, 2H), 7.58-7.53 (m, 2H), 4.99 (s, 2H), 2.61 (s, 6H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 161.5, 151.2, 150.3, 142.4, 134.5, 128.5 (2C), 128.4 (2C), 69.6, 50.7, 38.0 ppm.

4-[(5-Iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]-*N*-(propan-2-yl)benzene-1-sulfonamide (58) was synthesized according to the general procedure for the substitution by 5-iodouracil applied on intermediate **50**. **58** was obtained with 60 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.74 (s, 1H), 8.38 (s, 1H), 7.82-7.76 (m, 2H), 7.60 (d, J = 7.1 Hz, 1H), 7.50-7.44 (m, 2H), 4.96 (s, 2H), 3.20 (octett, J = 6.7 Hz,1H), 0.95 (d, J = 6.5 Hz, 6H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 161.6, 151.2, 150.3, 141.5, 128.3 (2C), 127.2 (2C), 69.5, 50.8, 45.7, 23.7 (2C) ppm.

***N*-Cyclopropyl-4-[(5-iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]benzene-1-sulfonamide (59)** was synthesized according to the general procedure for the substitution by 5-iodouracil applied on intermediate **51**. **59** was obtained with 61 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.75 (s, 1H), 8.39 (s, 1H), 7.93 (d, J = 1.5 Hz, 1H), 7.84-7.75 (m, 2H), 7.56-7.48 (m, 2H), 4.98 (s, 2H), 2.17-2.02 (m, 1H), 0.52-0.45 (m, 2H), 0.41-0.34 (m, 2H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 161.6, 151.2, 150.2, 141.8, 140.0, 128.3 (2C), 127.6 (2C), 69.5, 50.8, 24.6, 5.6 ppm.

***N*-tert-Butyl-4-[(5-iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]benzene-1-sulfonamide (60)** was synthesized in analogy to **56** from intermediate **54**. **60** was obtained with 45 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.73 (s, 1H), 8.37 (s, 1H), 7.84-7.78 (m, 2H), 7.55 (s, 1H), 7.49-7.43 (m, 2H), 4.96 (s, 2H), 1.09 (s, 9H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 161.6, 151.2, 150.2, 144.0, 141.1, 128.1 (2C), 127.0 (2C), 69.5, 53.8, 50.8, 30.2 ppm.

5-Iodo-1-{[4-(morpholine-4-sulfonyl)phenyl]methyl}-1,2,3,4-tetrahydropyrimidine-2,4-dione (61) was synthesized according to the general procedure for the substitution by 5-iodouracil applied on commercially available 4-[4-(bromomethyl)benzenesulfonyl]morpholine. **61** was obtained with 71 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.76 (s, 1H), 8.40 (s, 1H), 7.77-7.71 (m, 2H), 7.59-7.53 (m, 2H), 5.00 (s, 1H), 3.64-3.60 (m, 4H), 2.96-2.76 (m, 4H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 161.6, 151.2, 150.3, 142.8, 134.1, 128.6 (2C), 128.5 (2C), 69.6, 65.7 (2C), 50.8, 46.3 (2C) ppm.

Methyl 2-{4-[(5-iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methyl]benzenesulfonamido}acetate (62) was synthesized according to the general procedure for the substitution by 5-iodouracil applied on intermediate **52**. **62** was obtained with

43 % yield as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 11.74 (s, 1H), 8.38 (s, 1H), 8.23 (s, 1H), 7.80-7.73 (m, 2H), 7.53-7.45 (m, 2H), 4.96 (s, 2H), 3.70 (s, 2H), 3.49 (s, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C, TMS): δ 169.8, 161.6, 151.2, 150.2, 141.8, 140.3, 128.4 (2C), 127.3 (2C), 69.5, 52.2, 50.7, 44.2 ppm.

Biological testing. IC50 values were measured at Reaction Biology Corporation, Malvern, PA, USA with two to six replications using 10 concentrations starting at 10 μM or 100 μM with 3-fold dilution. The substrate used for the determinations was the (5-FAM/QXL™) FRET peptide (sequence = QXL® 520 - γ - Abu - Pro - Cha - Abu - Smc - His - Ala - Dab(5 - FAM) - Ala - Lys - NH_2 (Abu = 2-aminobutyric acid, Cha = β-cyclohexylalanine, Dab = diaminobutyric acid, Smc = S-methyl-L-cysteine, QXL® 520 = quencher, 5-FAM = 5-carboxyfluorescein, fluorescence dye), supplier: AnaSpec Inc., 34801 Campus Dr., Fremont, CA 94555, USA, product code: AS-60581-01). The buffer consisted of 50 mM HEPES at pH 7.5 with 10 mM $CaCl_2$ and 0.01 % Brij-35. 0.1 mg/ml BSA was added before use. As a control inhibitor GM6001 was used. IC_{50} calculation was done using the software GraphPad Prism7 with a four parameter least squares fit. Residual activities of the enzyme were measured in the same system at an inhibitor concentration of 10 μM in duplicates.

ASSOCIATED CONTENT

Supporting Information

NMR-Spectra, HPLC, HRMS, IC_{50} curves for all synthesized compounds, modeling description, molecular formula strings

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ABBREVIATIONS

COMU, ((1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium-hexafluorophosphate); DBPO, dibenzoyl peroxide; DIPEA, diisopropylethylamine; EEDQ, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; MeOH/MeOD, methanol / deuterated methanol; MMP, matrix metalloproteinase, TBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TOSMIC, p-toluenesulfonylmethyl isocyanide.

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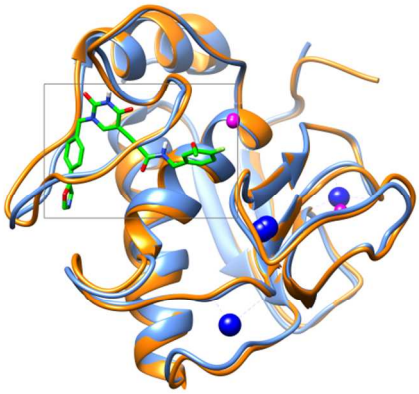

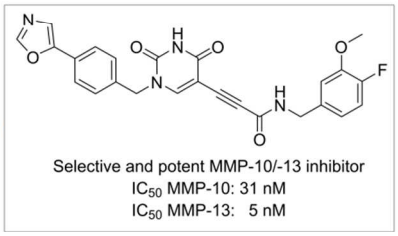

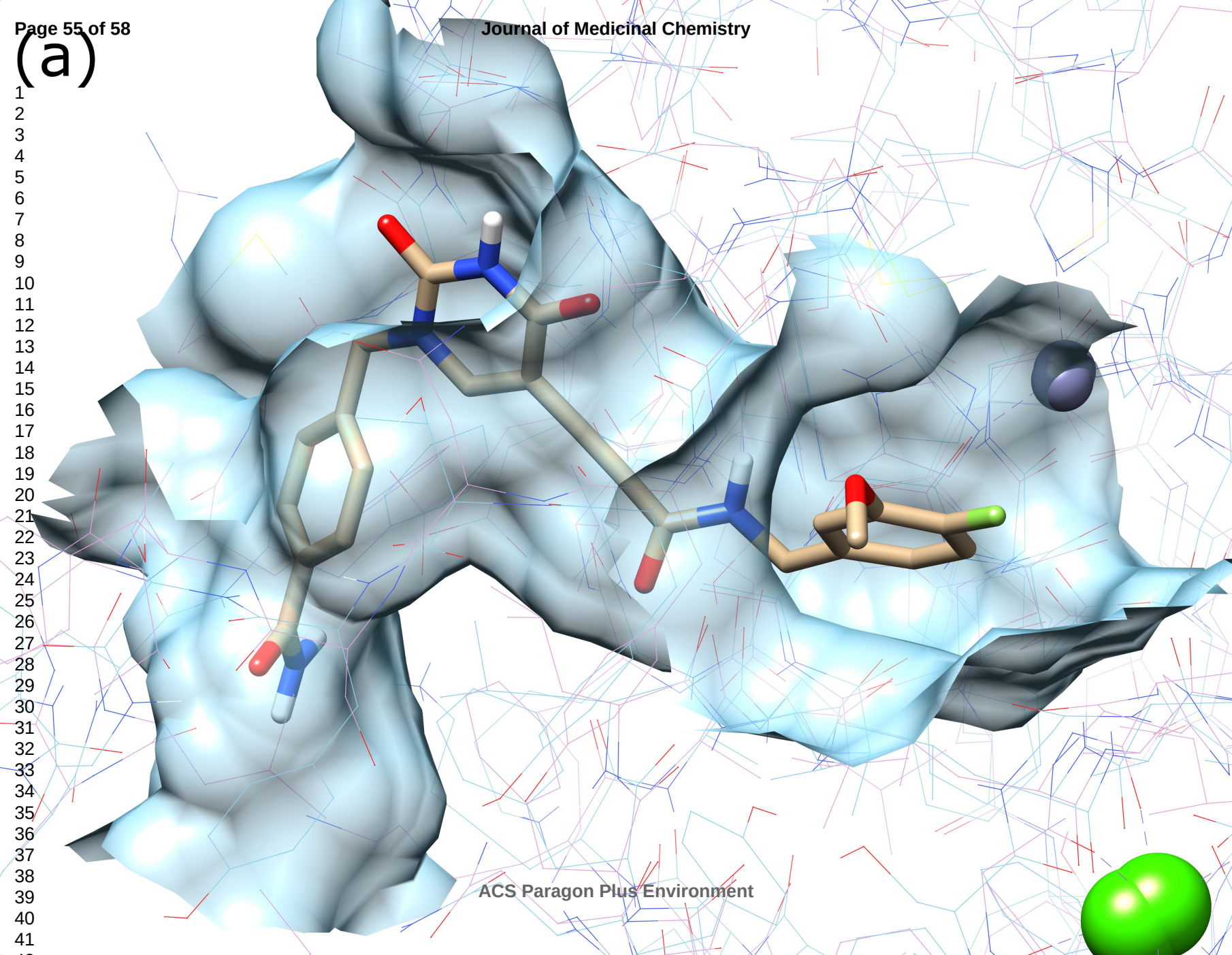
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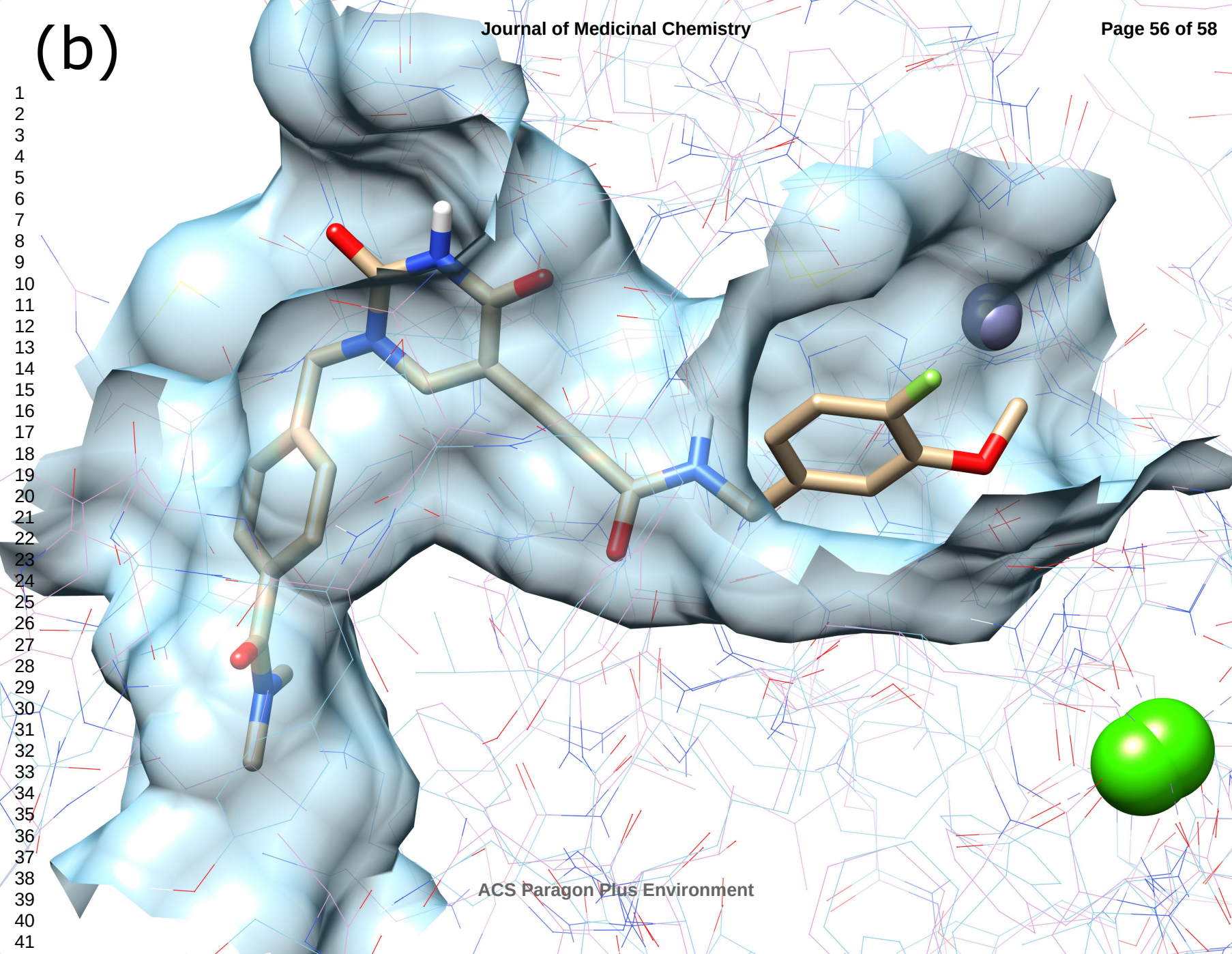
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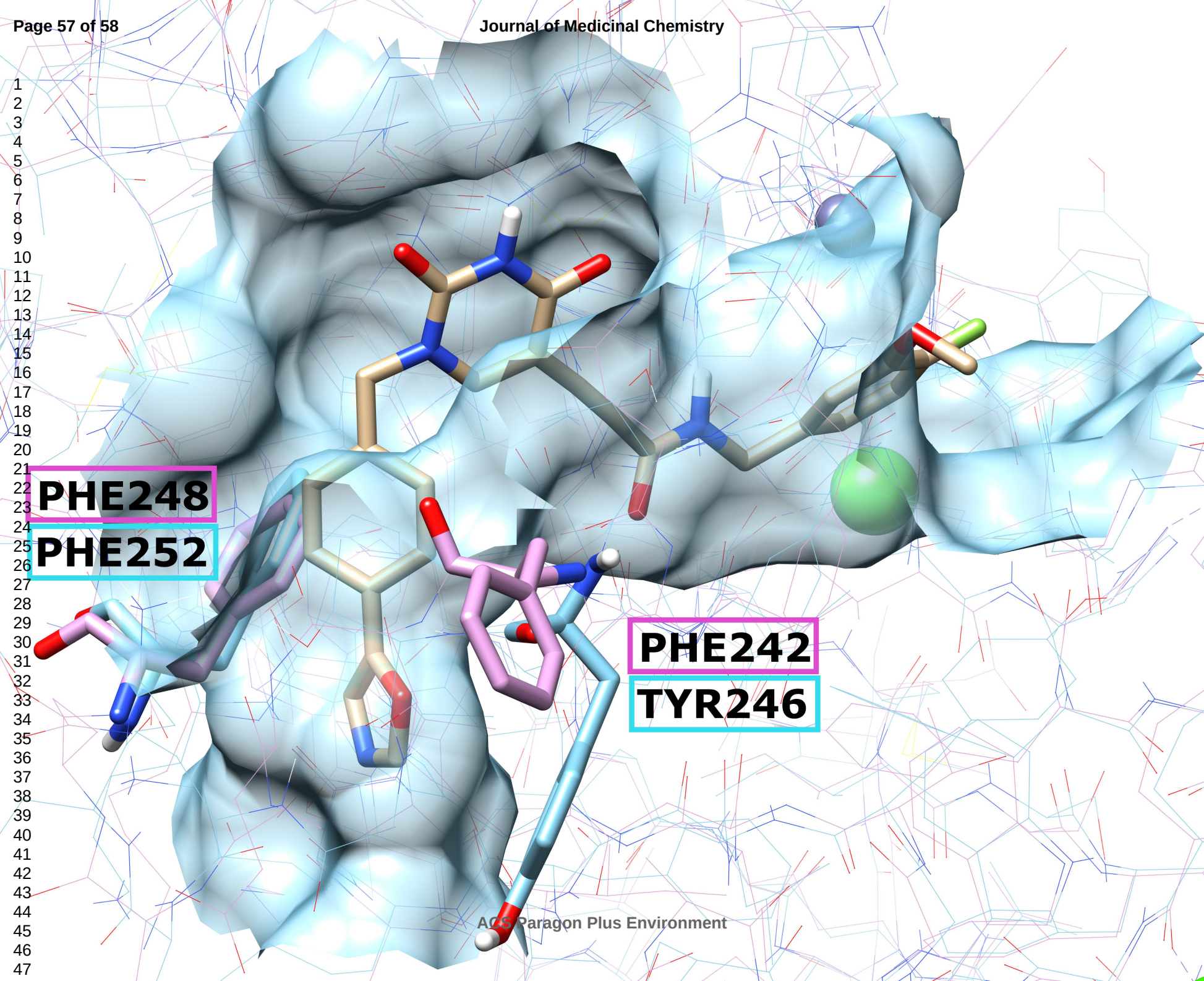
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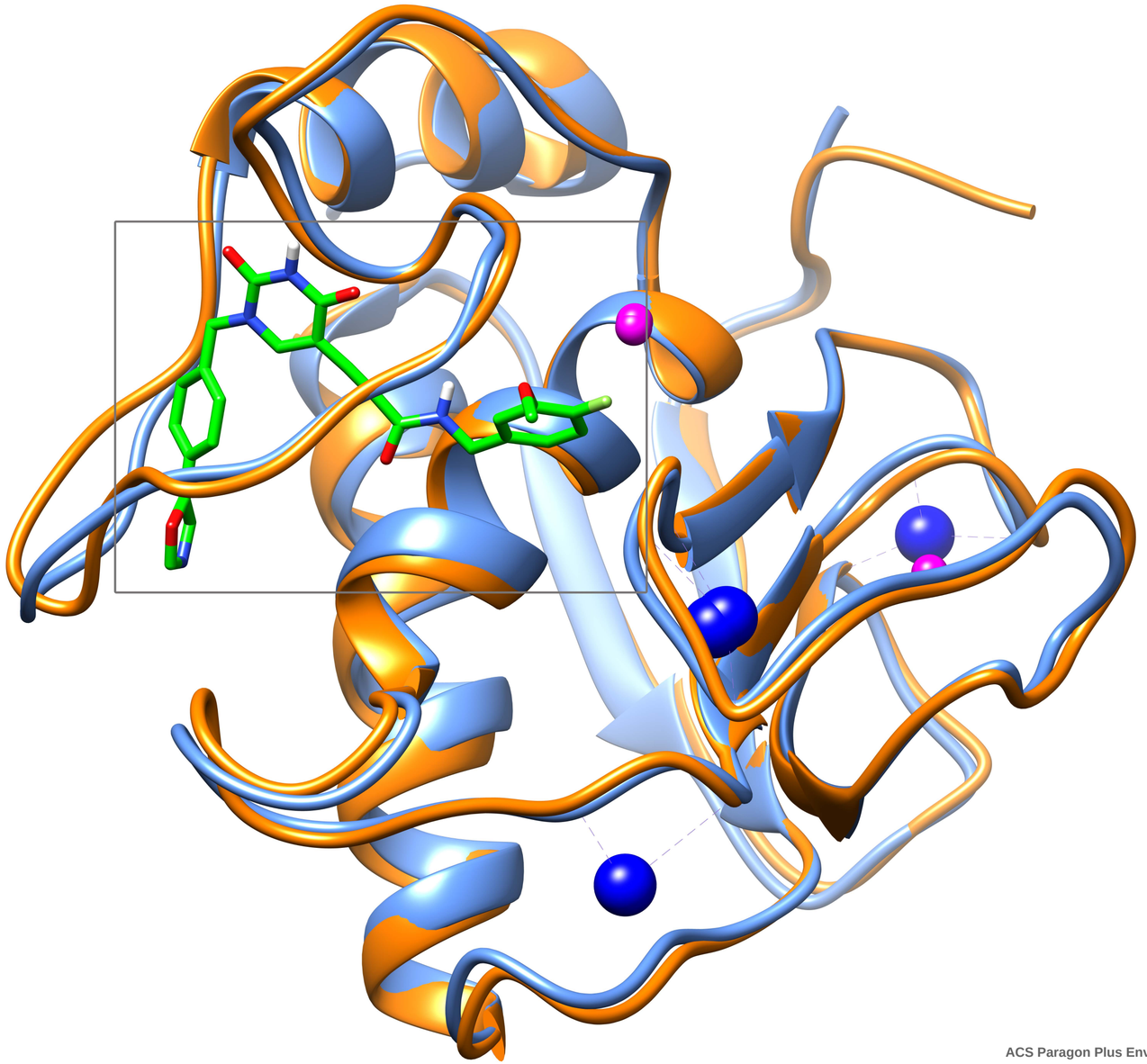

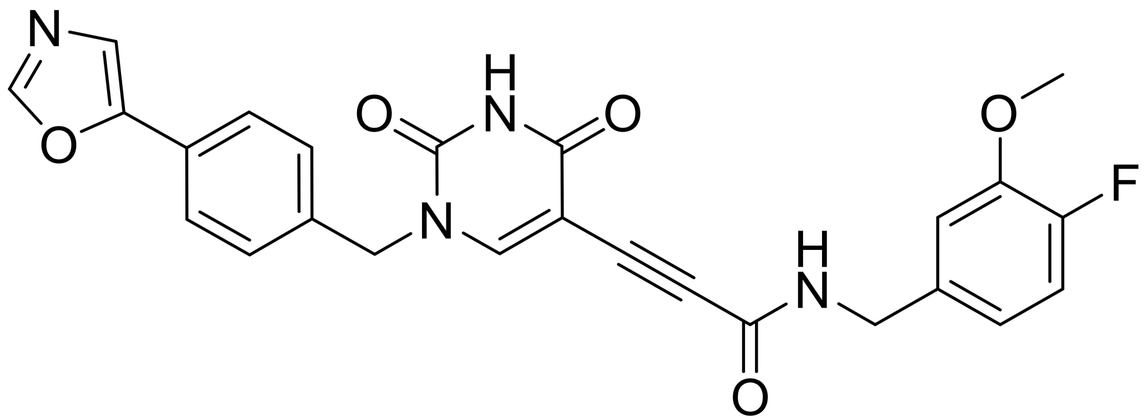

Selective and potent MMP-10/-13 inhibitor

IC_{50} MMP-10: 31 nM

IC_{50} MMP-13: 5 nM