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Article

Beyond Basicity: Discovery of Nonbasic DENV-2 Protease Inhibitors with Potent Activity in Cell Culture

Nikos Kühl, Mila M. Leuthold, Mira A. M. Behnam, and Christian D. Klein*

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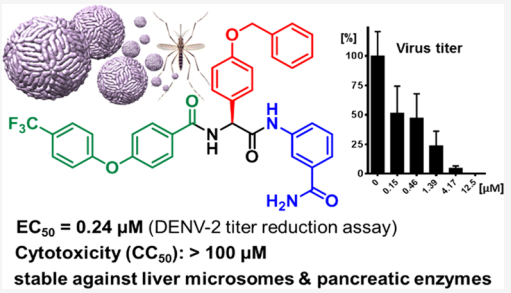
ABSTRACT: The viral serine protease NS2B-NS3 is one of the promising targets for drug discovery against dengue virus and other flaviviruses. The molecular recognition preferences of the protease favor basic, positively charged moieties as substrates and inhibitors, which leads to pharmacokinetic liabilities and off-target interactions with host proteases such as thrombin. We here present the results of efforts that were aimed specifically at the discovery and development of noncharged, small-molecular inhibitors of the flaviviral proteases. A key factor in the discovery of these compounds was a cellular reporter gene assay for the dengue protease, the DENV2proHeLa system. Extensive structure−activity relationship explorations resulted in novel benzamide derivatives with submicromolar activities in viral replication assays (EC_{50} 0.24 μM), selectivity against off-target proteases, and negligible cytotoxicity. This structural class has increased drug-likeness compared to most of the previously published active-site-directed flaviviral protease inhibitors and includes promising candidates for further preclinical development.



INTRODUCTION

Dengue is a mosquito-borne viral disease characterized by high fever, headache, muscle pain, and rash.[1,2] There are four distinct serotypes of dengue viruses (DENV) that cause dengue fever. Recovery from infection provides lifelong immunity against the involved serotype. Subsequent infections by other serotypes increase the risk of developing a severe form of dengue fever, which can threaten patients' lives.[3,4] The global incidence of dengue has grown dramatically in recent years.[3,4] This can be explained by population growth and density, inadequate public health, increased travel, and growing vector distribution.[1,5,6] Specific antiviral drugs against flaviviruses are not available, and the treatment remains supportive, focused on pain and fever control as well as fluid management.[7] A recently approved vaccine (Dengvaxia) performs differently in seropositive and seronegative patients, and its use is therefore highly restricted.[8,9]

Like other flaviviruses, dengue virus contains a positive-sense single-stranded RNA that is translated by host cell proteins into a single polyprotein. The viral serine protease NS2B-NS3 processes the viral polyprotein into functional structural and nonstructural proteins[10,11] and is therefore considered as a promising drug target.[10,12]

The hydrophilic domain of the membrane-associated nonstructural protein 2B is required as a cofactor for the protease activity in NS3. The protease has a strong preference for substrates with dibasic sequences in P_1 and P_2.[10,13,14] Since most of the known peptidic or peptide-hybrid inhibitors are based on those sequences, the existing chemotypes of inhibitors feature two or more basic functionalities, frequently arginine or arginine mimetics.[10,15−18] This accumulation of positive charge is problematic with respect to pharmacokinetics and off-target interactions with host proteases such as trypsin, thrombin, and generally with proteases from the blood clotting cascade.

Recently, we reported a novel class of compounds with lower molecular mass than previously published DENV protease inhibitors that incorporate a single cationic moiety.[19] The most active compounds contain a guanidine moiety (see Figure 1).[20] Off-target activities of this compound class were significantly reduced in comparison to peptidic inhibitors, but the pharmacokinetic properties (passive membrane permeation) remained problematic.

Efforts to increase activity in NS2B-NS3 drug discovery, and in medicinal chemistry in general, are frequently associated with increased molecular weight. In addition, inhibitors of flaviviral proteases frequently incorporate highly charged guanidino or similar functional groups because of the recognition preference of the enzyme for basic residues. Therefore, a challenge arises to reconcile the two opposing development aims of pharmacodynamic and pharmacokinetic optimization, where basicity can be considered a "friend and

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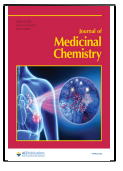


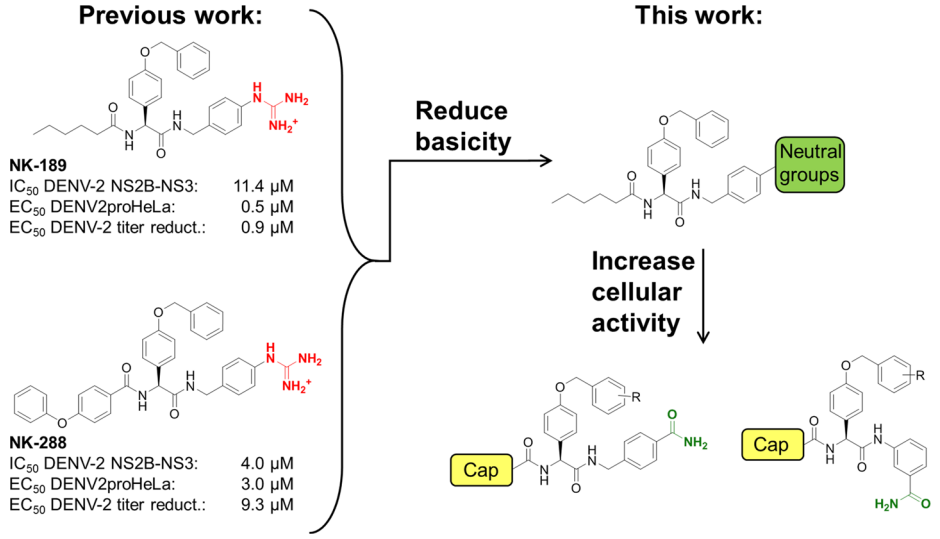
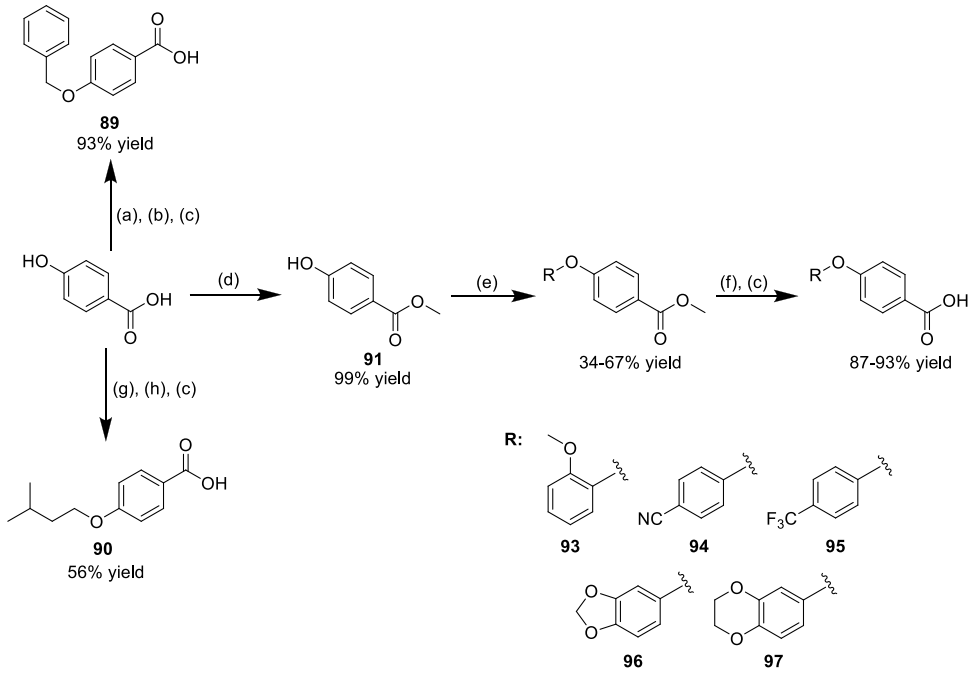


Figure 1. Recently published basic DENV NS2B-NS3 inhibitors[19] and the novel uncharged inhibitor series presented here.

Scheme 1. Synthesis of Various Cap Moieties[a]



[a](a) Benzyl bromide, K_2CO_3, DMF, rt to 80 °C, 72 h; (b) 2 M NaOH/MeOH (1:1), 50 °C; (c) HCl; (d) $SOCl_2$, MeOH, 0 °C to reflux, overnight; (e) respective phenylboronic acid, $Cu(OAc)_2$, pyridine, 4 Å sieves, DCM; (f) LiOH, THF/H_2O (2:1); (g) 1-bromo-3-methylbutane, K_2CO_3, DMF, 100 °C, overnight; (h) 2 M NaOH, 60 °C, overnight.

foe", respectively. This was also noticed in our own attempts, and the present work was therefore particularly aimed at a reduction of molecular size and polarity in order to approach the expected criteria for oral bioavailability, as described in the Lipinski rule of five and similar concepts.[21,22] Extensive structure–activity relationship (SAR) studies were conducted to identify structural elements that would allow the reduction of basicity while maintaining or improving the inhibitory activity. We herein present the synthesis, cellular structure–activity relationships, and in vitro pharmacokinetic and off-target evaluation of this inhibitor series.

We consider the application of a cellular DENV-2 protease reporter gene assay (DENV2proHeLa), which was described in detail before,[19] to be a crucial factor in the discovery and development of this compound class. The stable cotransfection of the DENV-2 NS2B-NS3 protease and an ODD-luciferase reporter construct allows high-throughput determination of intracellular DENV protease inhibition. Treatment of DENV2proHeLa cells with DENV-2 protease inhibitors decreases the *Renilla reniformis* luciferase signal significantly. In our previous work, we noticed a significant noncorrelation of biochemical assay DENV protease inhibitory values vs both antiviral efficacy and activity in the DENV2proHeLa reporter gene system. In contrast, the DENV2proHeLa system showed a relatively good correlation to antiviral efficacy in viral replication assays, the latter being considered the "gold

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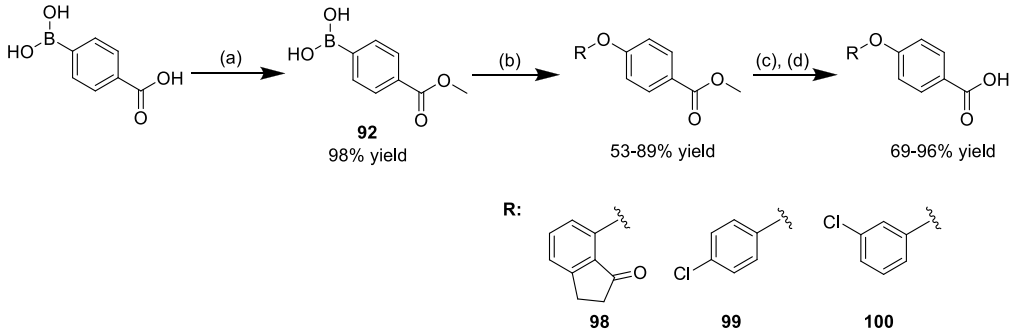
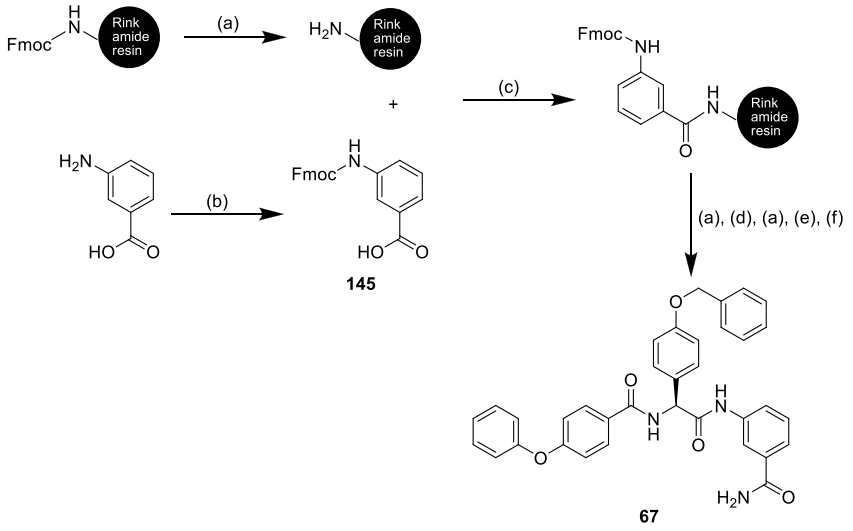
Scheme 2. Synthesis of Phenoxybenzoic Acid Cap Moieties[a]



[a](a) $SOCl_2$, MeOH, 0−50 °C, 5 h; (b) respective phenol derivative, $Cu(OAc)_2$, pyridine, 4 Å sieves, DCM; (c) LiOH, THF/H_2O (2:1); (d) HCl.

Scheme 3. Exemplary Solid-Phase Peptide Synthesis on Rink Amide Resin[a]



[a](a) Piperidine, DMF; (b) $NaHCO_3$, Fmoc-OSu, H_2O/ACN, 0 °C to rt; (c) COMU, TMP, DMF; (d) Fmoc-4-benzyloxy-L-phenylglycine, COMU, TMP, DMF; (e) 4-phenoxybenzoic acid, COMU, TMP, DMF; (f) TFA, H_2O, TIPS (95:4:1, v/v).

standard" for antiviral drug discovery. In other words, DENV2proHeLa appears to be a suitable surrogate for the tedious, time-consuming, and low-throughput viral replication assay. These observations, and the promising discovery of the lead compound with the DENV2proHeLa system, led us to favor this cellular assay as the primary SAR guidance tool in the present work.

RESULTS AND DISCUSSION

Chemistry. 4-Hydroxyphenylglycine was O-alkylated using alkyl halogenides as previously described.[16,19] Synthetic procedures and analytical data for the phenylglycine derivatives are provided in the Supporting Information (compounds **115**−**135**). 4-Phenoxyphenylglycine of compound **66** was synthesized by copper-mediated O-arylation with phenylboronic acid as described by Evans et al.[23] 4-Phenoxybenzoic acid derivatives were synthesized via the same procedure (Schemes 1 and 2): 4-Hydroxybenzoic acid and 4-carboxyphenylboronic acid were protected as methyl esters with thionyl chloride at increased temperature (compounds **91** and **92**). The O-arylation of the intermediates was performed with copper-catalyzed Chan−Evans−Lam coupling and can be conducted in air at room temperature.[23−26] LiOH in THF/H_2O (2:1) was used to cleave the methyl ester to yield the free carboxylic acid compounds. Cap **89** was synthesized by O-alkylation with benzyl bromide at 80 °C for 72 h and treatment with 2 M NaOH/MeOH (1:1) at 50 °C. In a similar reaction, 1-bromo-3-methylbutane was used for O-alkylation of **90** at 100 °C overnight.

The 4-benzyloxyphenylglycine derivatives were connected via amide bonds to various carboxylic acids and amines. The synthesis was executed by solid-phase peptide synthesis according to the Fmoc protocol or in solution. Synthetic routes are outlined in Schemes 3 and 4. Phenylglycine racemization was prevented by COMU/TMP conditions for all peptide coupling reactions on resin.[27] Benzamide compounds were synthesized using Rink amide resin as presented in Scheme 3. Amine building blocks were N-Fmoc protected in solution using Fmoc-OSu (see the Supporting Information, compounds **143**−**151**) before coupling to the resin under COMU/TMP conditions overnight.

Chlorotrityl chloride (CTC) resin was used for direct syntheses of compound **25** and the intermediates **101** and **102**. Compound **102** was coupled to the respective amine building block by peptide coupling in solution with HATU/TMP. The reaction was quenched with 5% TFA in DCM after two hours to avoid racemization of 4-benzyloxyphenylglycine (see Scheme 4). CTC resin was also used for syntheses of the hydroxyl compounds **9** and **10**. Therefore, the respective hydroxyl building blocks were N-Fmoc protected in solution

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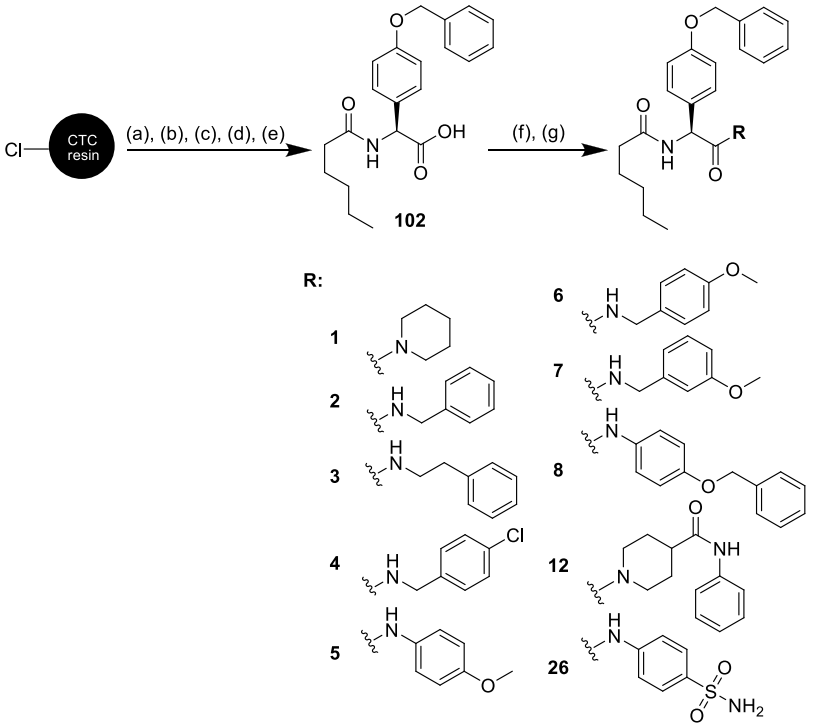
Scheme 4. Exemplary Solid-Phase Peptide Synthesis on Chlorotrityl Chloride Resin and Peptide Coupling in Solution[a]



[a](a) Fmoc-4-benzyloxy-L-phenylglycine, TMP, DMF; (b) DCM/MeOH/TMP (80:15:5, v/v); (c) piperidine, DMF; (d) hexanoic acid, COMU, TMP, DMF; (e) TFA, DCM; (f) HATU, respective amine derivative, TMP, DCM/DMF, 0 °C to rt; (g) TFA in DCM (5%, v/v), (7−92% yield).

with Fmoc-OSu (see the Supporting Information) before coupling to the solid support in pyridine overnight.

Analysis by chiral HPLC demonstrated that racemization during synthesis of the phenylglycine derivatives was negligible for this inhibitor set (see the Supporting Information for further details).

Synthesis of compounds **22** and **23** was described before.[19] Synthesis of precursors used for compounds **8**, **12**, and **26**, cap moieties, and other building blocks is described in detail in the Supporting Information.

Structure−Activity Relationships. Three series of compounds were evaluated in the DENV protease serotype 2 cellular reporter gene assay (DENV2proHeLa). This approach offers the opportunity to determine cellular activity, permeability, metabolic stability, and interaction with host factors in a single cellular assay. Another advantage of this assay is the use of a full-length construct of the DENV2 NS3 with its NS2B cofactor instead of a truncated protease with an artificial glycine-linker, which is used in most of the biochemical assays of DENV protease and in HTS campaigns.[10,28,29] Inhibitory activity assays were limited to one serotype. DENV serotype 2 was chosen since this serotype has the highest rate of severe manifestations like dengue shock syndrome.[30−32] Compounds **MB-53**,[16] **NK-189**,[19] and tolcapone[33] were included in the assays as reference inhibitors.

Furthermore, all compounds were tested against thrombin and trypsin in biochemical assays to evaluate their selectivity. The SAR explorations were focused on replacing the guanidine function with a less polar, and preferably noncharged, moiety. In addition, 4-benzyloxyphenylglycine derivatives and lipophilic cap moieties were evaluated.

Nonbasic Guanidine Replacements. The primary goal of this work was to identify a less basic or neutral alternative for the guanidine moiety while maintaining or increasing cellular antiviral activity and selectivity against off-targets. As outlined in Table 1, a variety of nonbasic replacements for the guanidinophenyl moiety were studied, based on the lead compound with a 4-benzyloxyphenylglycine moiety and a hexanoic acid cap. Whereas the 4-chlorobenzyl (compound **4**), 4-methoxyphenyl (**5**), and unsubstituted benzyl (**2**) derivatives had no relevant activity, the 4-methoxybenzyl (**6**), 4-hydroxyphenethyl (**10**), 4-(phenyl)methanol (**9**), and 4-acetamidobenzyl (**21**) derivatives had activities (EC_{50}) between 10 and 20 μM. Moving into the structural vicinity of the guanidino group, para- and meta-substituted benzamides yielded one-digit micromolar inhibition values and were therefore considered potential noncharged guanidine replacements at this position (compounds **14−19**). This represents an analogy to earlier reports on *meta*-benzamide derivatives described as guanidine isosters to achieve binding in the S1 pockets of factor Xa[34] and the tissue factor/factor VIIa complex.[35,36] The urea, hydrazide, and hydroxamic acid analogs **22**, **23**, and **24** are also similar to the guanidine moiety, but only minor inhibition was detected. No relevant activity was detected for the benzenesulfonamide (**26**) and benzoic acid compound (**25**). Hence, no negative charge and no large groups at this position like 4-benzyloxyphenyl (**8**) and *N*-phenylpiperidine-4-carboxamide (**12**) were tolerated. The cycloalkyl derivatives **11** and **13** demonstrated low activity, except piperidine **1** with an EC_{50} of around 13 μM. Cytotoxic effects were low or absent, with the exception of compound **3**, which could therefore not be studied in cellular assays.

Merging of Fragments. The benzamides, as the most active fragments, were merged with different 4-benzyloxyphenylglycine derivatives and different cap moieties to further increase their activity. 4-Benzamide and 3-benzamide deriva-

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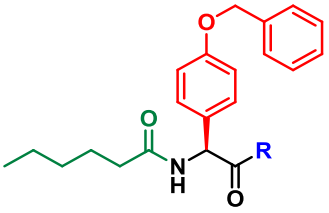
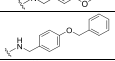
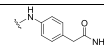
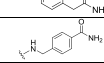
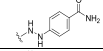
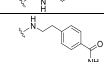
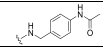
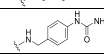
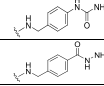
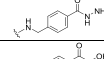
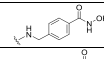
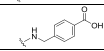
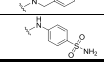
Table 1. Inhibitory Activity of Compounds with Different Nonbasic Moieties in the Cellular DENV Protease Reporter Gene Assay (DENV2proHeLa) and Cytotoxicity in HeLa Cells



Cpd.	R	DENV2proHeLa[a] % (12.5 μM)	DENV2proHeLa[a] EC_{50} (μM)	CC_{50} (μM)[b]
1		58 ± 7	13 ± 1	29 ± 3
2		n.i.	n.d.	> 50
3		n.d.	n.d.	< 12.5
4		14 ± 8	n.d.	> 50
5		10 ± 4	n.d.	> 25
6		3 5± 7	28 ± 2	> 50
7		n.i.	n.d.	> 50
8		n.i.	n.d.	> 50
9		48 ± 7	18 ± 1	> 50
10		58 ± 4	10 ± 0	> 25
11		28 ± 2	n.d.	> 50
12		35 ± 9	20 ± 3	> 50
13		29 ± 6	n.d.	> 50
14		58 ± 0	7.0 ± 0.5	> 12.5
15		76 ± 2	4.6 ± 0.3	> 50
16		13 ± 9	n.d.	> 50

Cpd.	R	DENV2proHeLa[a] % (12.5 μM)	DENV2proHeLa[a] EC_{50} (μM)	CC_{50} (μM)[b]
17		47 ± 1	n.d.	> 50
18		66 ± 2	6.3 ± 0.2	> 100
19		60 ± 2	8.7 ± 0.1	> 25
20		62 ± 7	7.7 ± 0.4	> 100
21		55 ± 8	14 ± 1	> 100
22[19]		24 ± 9	n.d.	> 50
23[19]		31 ± 7	n.d.	> 50
24		40 ± 2	n.d.	> 50
25		14 ± 7	n.d.	> 50
26		n.i.	n.d.	> 50
MB-53[16]		44 ± 9	19 ± 2	> 50
NK-189[19]		80 ± 5	0.49 ± 0.08	> 50
tolcapone[33]		59 ± 7	7.1 ± 0.8	> 25

[a]Values against the DENV serotype 2 protease in the reporter gene assay in HeLa cells. [b]Measured in HeLa cells. If inhibition ≤ 10%, then there is no inhibition (n.i.). n.d. = not determined. All measurements were carried out in triplicate.

tives were evaluated (Tables 2 and 3, respectively). Whereas polar caps like 2-(2-methoxyethoxy)acetic acid (compound **47**), 6-aminohexanoic acid (compound **49**), or 4-(aminomethyl)benzoic acid (**50**) decreased the activity, long alkyl chains and ring systems maintained or even increased it. Thiophene ring systems, 4-phenoxy- and 4-benzyloxybenzoic acid cap moieties, led to potent inhibitors. Secondary amines like the alkyl compound (**46**) and the benzyl derivative (**53**) decreased the cellular activity, possibly due to the additional positive charge. Further extension of the 4-benzyloxy residue was not well-tolerated. Only the 3-methoxy-, 4-(trifluoromethyl), and 1,3-dichloro derivatives had comparable activities to compound **18**. L-Configuration at the Phg chiral center is preferred, as demonstrated before for the basic derivatives.[19] Removal of the benzyloxy fragment (R_2 in compounds **27** and **29**) and its exchange to a 4-trifluoromethyl moiety or an amino acid exchange to phenylalanine (compound **30**) resulted in loss of activity.

Compound **57** caused 50% inhibition of DENV protease activity at 1 μM but also showed relevant cytotoxicity (CC_{50}, 18 μM; SI, 18). To overcome this problem, meta-substituted derivatives were evaluated (Table 3). Compound **67** in contrast to compound **57** was not cytotoxic up to 100 μM and demonstrated slightly higher activity. Similarly, all other meta-substituted derivatives caused no relevant cytotoxicity and had a significantly higher selectivity index than the previously published series of compounds.[19] Most of the evaluated phenoxyphenyl or biphenyl caps resulted in high inhibitory activities with EC_{50} values at about 0.6−2 μM (except compound **86**), whereas the 2-naphthoic acid cap (**62**) and the 2,3-dihydrobenzo[*b*][1,4]dioxine-6-carboxylic acid cap (**63**) resulted in lower activities around 5 μM. The 4-benzoylbenzoic acid capped compound (**77**) as well as compounds **67** and **72** revealed high nanomolar inhibition and an excellent selectivity index (SI > 140). Substitution of the phenoxy group of compound **67** was mostly tolerated: 2-Methoxy, 4-cyano, 4-chloro, 3-chloro, and 4-trifluoromethyl caps resulted in comparable to 3-fold higher EC_{50} values relative to compound **67**. 4-(Benzo[*d*][1,3]dioxol-5-yloxy)-benzoic acid (**74**) showed about 2-fold higher activity compared to next homolog 4-(2,3-dihydrobenzo[*b*][1,4]-dioxin-6-yloxy)benzoic acid (**75**). The (morpholine-4-carbonyl)benzoic acid derivatives **79** and **80** were designed in analogy to compound **77** with a polar morpholine moiety, but the activity decreased significantly. Compound **66** contains a 4-phenoxyphenylglycine core, which slightly decreased the

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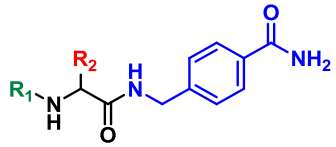
Table 2. Inhibitory Activity of 4-Benzamide Compounds with Different 4-Benzyloxyphenylglycine Derivatives and Different Cap Moieties in the Cellular DENV Protease Reporter Gene Assay (DENV2proHeLa) and Cytotoxicity in HeLa Cells



Cpd.	R_1	R_2	DENV2proHeLa[a] % (12.5 μM)	DENV2proHeLa[a] EC_{50} (μM)	CC_{50} (μM)[b]
27			15 ± 5	n.d.	> 50
28			31± 7	n.d.	> 50
29			n.i.	n.d.	> 100
30			n.i.	n.d.	> 50
18			66 ± 2	6.3 ± 0.2	> 100
31			35 ± 3	n.d.	> 50
32			53 ± 4	9.8 ± 0.6	> 100
33			42 ± 7	n.d.	> 50
34			n.i.	n.d.	> 12.5
35			43 ± 8	n.d.	> 50
36			61 ± 10	7.3 ± 0.5	> 100
37			71 ± 3	8.3 ± 0.6	> 50
38			19 ± 2	n.d.	> 50
39			16 ± 9	n.d.	> 50
40			33 ± 5	n.d.	> 50

Cpd.	R_1	R_2	DENV2proHeLa[a] % (12.5 μM)	DENV2proHeLa[a] EC_{50} (μM)	CC_{50} (μM)[b]
41			45 ± 8	n.d.	> 50
42			38 ± 8	14 ± 2	> 100
43			52 ± 5	7.9 ± 0.3	> 50
44			56 ± 6	n.d.	> 50
45			47 ± 2	11 ± 1	> 25
46			19 ± 9	n.d.	> 50
47			27 ± 3	n.d.	> 50
48			n.i.	n.d.	> 50
49			14 ± 1	n.d.	> 50
50			24 ± 2	29 ± 4	> 50
51			57 ± 3	12 ± 1	> 50
52			34 ± 14	n.d.	> 50
53			36 ± 3	n.d.	> 50
54			72 ± 4	8.4 ± 0.7	> 50
55			71 ± 4	6.6 ± 0.2	> 50
56			63 ± 2	6.2 ± 0.1	> 100
57			82 ± 4	1.0 ± 0.1	18 ± 1
58			64 ± 9	4.0 ± 0.2	> 25
59			25 ± 13	n.d.	> 50

[a]Values against the DENV serotype 2 protease in the reporter gene assay in HeLa cells. [b]Measured in HeLa cells. If inhibition ≤ 10%, then there is no inhibition (n.i.). n.d. = not determined. All measurements were carried out in triplicate.

activity compared to the 4-benzyloxyphenylglycine derivatives. 5-(2,5-Dimethylphenoxy)-2,2-dimethylpentanoic acid, the cap in compound **82**, is a known drug (INN: Gemfibrozil) for treatment of hyperlipidemia and hypertriglyceridemia.[37] Phenylpiperidine compound **87** revealed low inhibition, which could be explained with the basic nature of the cap moiety and the expected lower membrane penetration of the compound. Other ring exchanges in the cap moiety to heterocycles like furan (compound **85**) and morpholine (compound **88**) and substitution at the para position (compound **84**) were well-tolerated.

Inhibitory Activity against Isolated DENV-2 and WNV Proteases. Surprisingly, only low to moderate inhibition was observed against the isolated DENV-2 and WNV proteases in biochemical assays (see the Supporting Information for details). This inconsistency was observed for other DENV protease inhibitors before.[19,38−41] We interpret this phenomenon as a consequence of two factors: first, the artificial composition of the biochemical assay. The assay is usually performed at basic pH values of 9.0 or 8.5, and high concentrations of polyols are needed for adequate activity of the protease.[10,28,42] Addition of physiological salt concentrations to the assay buffer leads to a significant decrease in proteolytic activity[43,44] and lowers the interaction with the cofactor NS2B.[45] Furthermore, other assay components could lead to false negative results against isolated DENV and WNV proteases as observed previously for nonionic detergents.[46−48] Upon exchange of the nonionic detergent Brij 58 to the ionic detergent CHAPS, we observed increased inhibitory activity of the compounds at the isolated targets (Table S2). The most active derivatives showed potency in the low micromolar IC_{50} range against DENV-2 and WNV proteases. Both viral proteases have been described before as sensitive toward buffer composition and detergents in particular.[46,47] Li et al. described a ZIKV split luciferase complementary assay, in which nonionic detergents interfered strongly with the signal and revealed inhibitory effects.[49] It may be hypothesized that certain structural features, like the aliphatic hexadecyl chain in Brij 58, occupy lipophilic binding sites on the target proteins and thereby interfere with target recognition of the present

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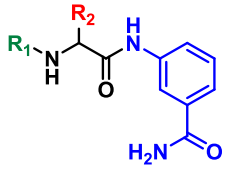
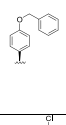
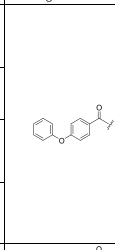
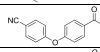
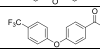
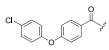
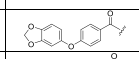
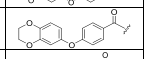
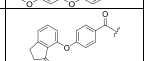
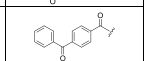
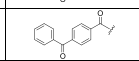
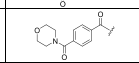
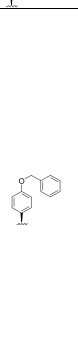
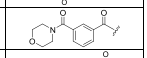
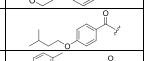
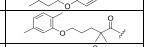
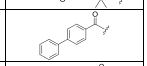
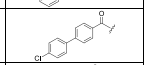
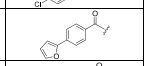
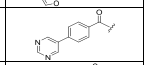
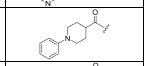
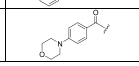
Table 3. Inhibitory Activity of 3-Benzamide Compounds with Different 4-Benzyloxyphenylglycine Derivatives and Different Cap Moieties in the Cellular DENV Protease Reporter Gene Assay (DENV2proHeLa) and Cytotoxicity in HeLa Cells



Cpd.	R_1	R_2	DENV2proHeLa[a] % (12.5 μM)	DENV2proHeLa[a] EC_{50} (μM)	CC_{50} (μM)[b]
60			65 ± 2	2.4 ± 0.1	> 50
15			76 ± 2	4.6 ± 0.3	> 50
61			66 ± 6	2.2 ± 0.3	> 12.5
62			71 ± 3	4.5 ± 0.5	> 100
63			69 ± 14	5.4 ± 0.3	> 100
64			71 ± 9	2.2 ± 0.2	> 50
65			73 ± 3	2.1 ± 0.1	> 100
66			73 ± 2	2.9 ± 0.1	> 50
67			83 ± 1	0.73 ± 0.04	> 100
68			84 ± 6	1.7 ± 0.3	> 100
69			77 ± 4	2.2 ± 0.1	> 100
70			79 ± 3	1.0 ± 0.0	> 100
71			76 ± 2	1.2 ± 0.2	> 100
72			86 ± 6	0.58 ± 0.03	96 ± 8
73			87 ± 6	1.0 ± 0.1	> 100

Cpd.	R_1	R_2	DENV2proHeLa[a] % (12.5 μM)	DENV2proHeLa[a] EC_{50} (μM)	CC_{50} (μM)[b]
74			78 ± 1	1.5 ± 0.2	> 100
75			61 ± 1	3.8 ± 0.3	> 100
76			57 ± 8	3.9 ± 0.6	> 100
77			76 ± 5	0.39 ± 0.02	> 100
78			79 ± 1	1.2 ± 0.1	> 100
79			40 ± 14	15 ± 1	> 100
80			35 ± 10	23 ± 4	> 100
81			49 ± 3	n.d.	> 50
82			82 ± 6	1.7 ± 0.2	> 25
83			79 ± 4	1.7 ± 0.3	> 100
84			78 ± 8	1.2 ± 0.1	> 25
85			76 ± 6	1.7 ± 0.1	> 50
86			59 ± 7	8.1 ± 0.9	> 50
87			46 ± 7	n.d.	> 50
88			79 ± 6	2.2 ± 0.4	> 100

[a]Values against the DENV serotype 2 protease in the reporter gene assay in HeLa cells. [b]Measured in HeLa cells. If inhibition ≤ 10%, then there is no inhibition (n.i.). n.d. = not determined. All measurements were carried out in triplicate.

series of compounds. The inhibitors BP2109 and BP13944 consist of long alkyl chain residues, and their inhibition against the NS3 domain was proven by selection for resistance experiments.[39,40] This hypothesis, however, is difficult to align with the substrate-competitive binding mode shown for selected compounds (see below). Second, the cellular DENV2proHeLa assay incorporates a full-length and not a truncated viral protease with covalent a Gly-Ser NS2B linker as in most of the biochemical studies. The transmembrane helices of NS2B that anchor the active protease complex to the endoplasmic reticulum are missing in the isolated protease construct, and the cofactor is covalently linked to the protease domain by an artificial linker sequence.[44] Furthermore, an intracellular or subcellular accumulation of the compounds may lead to increased activity in cellular assay systems, as pointed out by others before.[38] Taken together, the biochemical assay, with its presence of non-natural detergents and buffers, may not resemble the plasticity and functional behavior of the full-length protease complex within its in vivo environment, and the predictive value of biochemical assays for antiviral efficacy in cells appears to be limited. In contrast, we recently found a relatively good correlation between efficacies in the cellular DENV-2 reporter gene (DENV2proHeLa) and the "gold standard" DENV-2 titer reduction assays.[19]

Selectivity against Off-Target Proteases and Binding Mode. Several off-target serine proteases, in particular thrombin and trypsin, have a recognition preference for basic moieties in S1. They are therefore potential off-targets, and their interaction with flaviviral protease inhibitors, who have a similar property profile, should be studied. To determine the activity of the compound against potential off-targets, biochemical thrombin and trypsin assays were performed. None of the compounds had activity against thrombin and trypsin at compound concentrations of 25 and 50 μM, respectively (see Table S3).

IC_{50} values of compounds **67**, **71**, **77**, and **84** were determined against the isolated DENV-2 protease at different substrate concentrations, resulting in the Cheng−Prusoff plots provided in Figure S1. All plots indicate a competitive inhibition of the viral protease. Compounds **67**, **71**, **77**, and **84** have K_i values of 6.1, 6.3, 3.5, and 6.9 μM, respectively. Additionally, Michaelis−Menten studies with different concentrations of compounds **67** and **84** were performed (Figure S3). Both compounds showed no significant change for the

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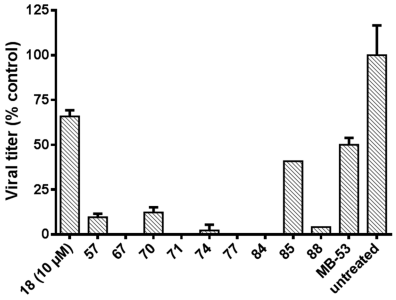
V_{max} value but an increase of the K_m value, presenting the behavior of competitive inhibitors. The K_i values derived from Michaelis−Menten plots for compounds **67** and **84** were 5.2 and 4.1 μM, respectively.

Tryptophan quenching experiments[48] were performed with compounds **56** and **85**, which both incorporate caps with a significant quenching effect. Both compounds were active in the cellular assay (DENV2proHeLa) in the low micromolar range. The addition of the compounds to the isolated DENV-2 protease resulted in a concentration-dependent quenching of the fluorescence of tryptophan residues of the protease (Figure S4). The active site inhibitor aprotinin restored fluorescence, which is interpreted to result from displacement of **56** and **58**, indicating a binding of the compounds in the same region (active site) as aprotinin. The 2,2′-bithiophene-5-carboxylic acid cap (compound **56**) was used as a quenching moiety for this application before,[16,19] whereas the 4-(furan-2-yl)benzoic acid cap (compound **85**) is newly described here.

Antiviral Activity in Virus Titer Reduction Assay. To confirm the high cellular activity of the compounds in the DENV2proHela assay, selected compounds and reference inhibitor **MB-53** were further tested in a DENV2 titer reduction assay. Huh-7 cells were infected with DENV2 (MOI of 1) and treated with various concentrations of the compounds as indicated. The supernatants were collected at 48 h post-infection and analyzed by a plaque assay. The tested compounds revealed high reduction of the viral titer even at the low screening concentration of 12.5 μM (see Figure 2).



Cmp.	inhibition [%]	cytotoxicity [%]
18*	34	< 20.0
57	90	61.4
67	100	< 20.0
70	88	< 20.0
71	100	< 20.0
74	98	< 20.0
77	100	< 20.0
84	100	< 20.0
85	59	< 20.0
88	96	< 20.0
MB-53	50	< 20.0
untreated	0	n.d.

*measured at 10 µM

Figure 2. Inhibitory activity and cytotoxicity of selected compounds and reference compound **MB-53** at a concentration of 12.5 μM in the DENV-2 virus titer reduction assay.

The inhibitors did not show cytotoxicity in Huh-7 cells at this concentration except compound **57**. It should be noted that the most active compounds inhibit up to 100% of the viral titers at the tested concentration. Compounds **18** and **85** demonstrated lower activity than in the DENV2proHeLa assay, whereas the high activity of the other inhibitors is in accordance with their DENV2proHeLa screening results.

Antiviral dose−response curves were obtained for the most active derivatives (Figure 3). Residual plots, showing the differences between observed values and curve fitting results for all viral titer reduction curves, are provided in the Supporting Information (Figure S5). All tested compounds resulted in dose-dependent reduction of viral titers, with EC_{50} values ranging from 0.24 to 2.2 μM (Table 4). Unsubstituted compound **67** as well as the 4-cyano compound (**70**) had EC_{50} values of 0.66 μM, whereas the 4-(benzo[*d*][1,3]dioxol-5-yloxy)benzoic acid compound (**74**) showed 3-fold lower activity. A 4-trifluoromethyl substitution (**71**) increased the activity more than 2-fold to a very promising EC_{50} of 0.24 μM, and this compound is one of the most active viral protease inhibitors in a flaviviral titer reduction assay reported to date. The 4-benzoylbenzoic acid (compound **77**), 4′-chlorobiphenyl-4-carboxylic acid (compound **84**), and 4-morpholinobenzoic acid caps (compound **88**) led to EC_{50} values of 0.85 μM. The EC_{90} values in the low micromolar range provide further evidence for the high antiviral activity of the compounds.

Membrane Permeability and Metabolic Stability. By using the precoated trilayer parallel artificial membrane permeability assay (PAMPA),[50,51] passive membrane permeability of selected compounds and references was evaluated (Table 5). The passive permeability for the references was in accordance with literature values.[50] Whereas compound **18** showed no permeability, compounds **57**, **67**, **70**, **71**, **74**, **77**, **84**, **85**, and **88** revealed significant passive permeability. The tested set of benzamide compounds showed significantly higher passive membrane penetration than the previously described guanidine compound set, which explains the higher intracellular antiviral activity of the benzamides.[19] Compounds **67**, **77**, and **88** showed even higher passive membrane penetration than guanidine compound **NK-189**, which previously had shown the most promising value. The measured passive permeability values are near the range of approved drugs.[50]

An important factor to consider during the early stages of drug discovery is compound stability against liver and pancreatic enzymes. Many compounds with promising in vitro activities never become drugs due to poor metabolic profiles. The metabolic stability of compounds **67**, **71**, **77**, **84**, and **88** as well as **NK-189** and reference compound testosterone was assessed using liver microsomes from rats. Liver microsomes contain phase 1 metabolic enzymes that catalyze typical phase 1 metabolic reactions, such as reduction, oxidation, and hydrolysis of compounds.[52] As described before,[16,18,53] samples were incubated for 60 min at 37 °C, and the depletion of the parent compound was monitored (Figure 4). Testosterone ($t_{1/2}$ = 16 min) and compounds **NK-189** ($t_{1/2}$ = 55 min) and **88** ($t_{1/2}$ = 27 min) showed significant depletion of the parent compound, whereas compounds **71** and **84** were stable over the whole test period. Except compound **88**, the newly described benzamide inhibitors had significantly higher stability against liver microsomes than compound **NK-189**. All assessed inhibitors demonstrated remarkable stability compared to previously published DENV protease inhibitors.[18]

The newly described structures include carboxamide bonds, which are prone to cleavage by pancreatic enzymes such as α-chymotrypsin and trypsin upon oral administration. Therefore, the stability of selected compounds (**NK-189**, **67**, **71**, **77**, **84**, and **88**) was assessed against those enzymes as previously reported.[53,54] Compound **MB-53** was examined before[53] and was included as a reference compound. Compounds were incubated with the respective enzyme in buffer for 120 min at 37 °C, and the depletion of the parent compound was monitored. Except for reference compound **MB-53** and **88**, all compounds demonstrated stability against bovine trypsin (maximum 25% depletion of parent compounds over 120 min) (Figure S6). Reference compound **MB-53** showed comparable half-life time against α-chymotrypsin to previous published data ($t_{1/2}$ = 25 min).[53] 70−80% of compounds **67**, **71**, **77**, and **84** remained after 120 min incubation with α-chymotrypsin (Figure S7). Compounds **NK-189** and **88** were less stable (approximately 40−50% compound cleavage). The incorporation of a 4-morpholinobenzoic acid cap and 4-

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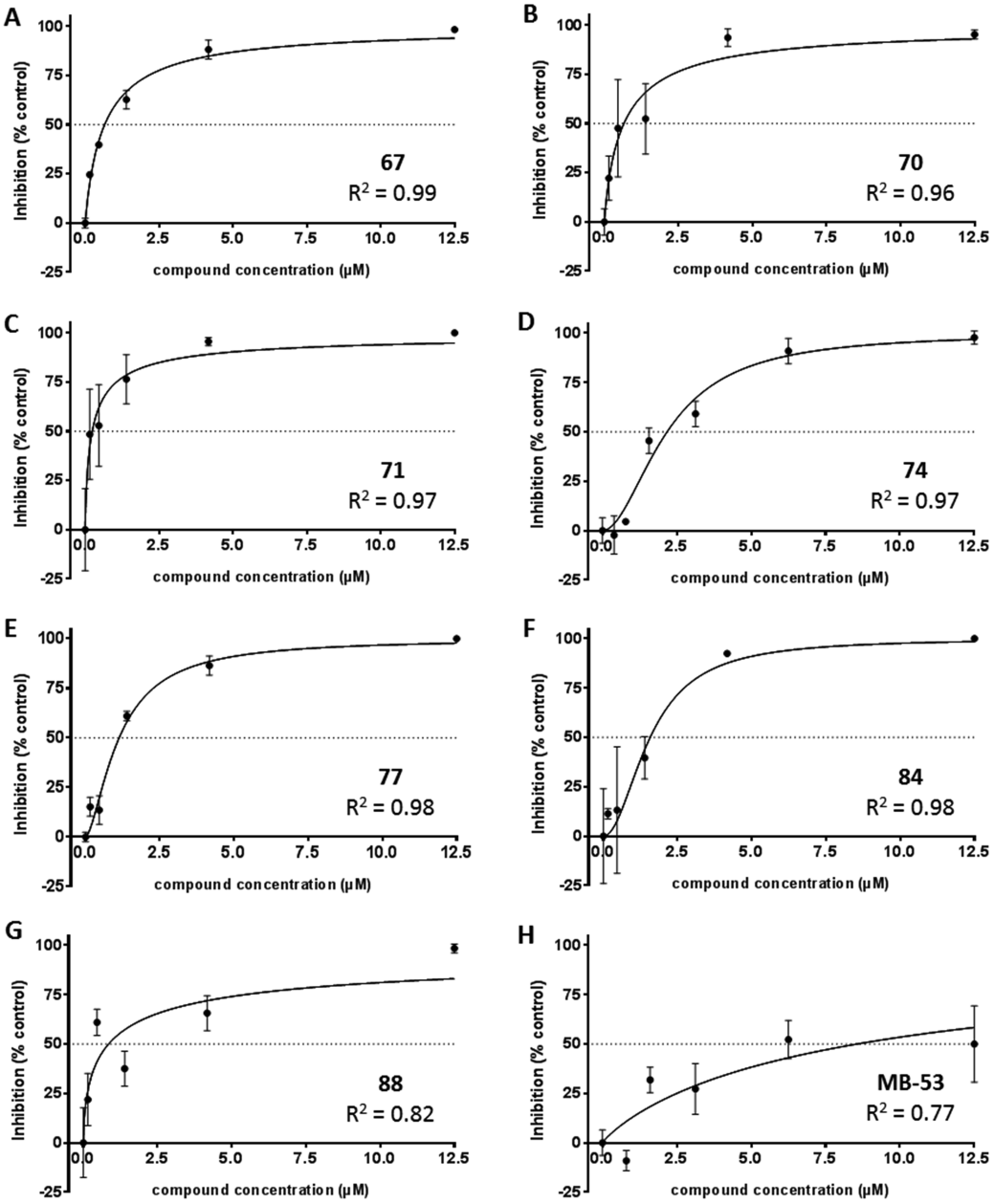

Figure 3. Viral titer reduction assay: concentration-dependent DENV-2 titer reduction for selected compounds, including reference compound **MB-53** (chart H).

Table 4. EC_{50} and EC_{90} Values for Selected Compounds in DENV-2 Titer Reduction Assay[a]

compound	EC_{50} [μM]	EC_{90} [μM]
67	0.66 ± 0.08	7.0 ± 0.1
70	0.66 ± 0.17	8.0 ± 0.1
71	0.24 ± 0.07	4.8 ± 0.1
74	2.2 ± 0.2	6.8 ± 0.1
77	1.1 ± 0.2	4.6 ± 0.1
84	1.6 ± 0.2	4.7 ± 0.1
88	0.85 ± 0.51	15 ± 0.2
MB-53[16]	8.6 ± 3.5	n.d.

[a]n.d. = not determined. All measurements were carried out in duplicate.

phenylguanidine appears to decrease the stability of the presented compound series against chymotrypsin.

CONCLUSIONS

The aim of the present work was to increase the drug-likeness and cellular activity of DENV NS2B-NS3 inhibitors, with the activity in the DENV2proHeLa reporter gene assay as the main SAR guidance tool. The charged guanidine moiety, which is present in peptidic and nonpeptidic compounds reported before,[15−17,55−58] was successfully replaced by nonbasic groups. Optimization of the substituents on the P_1 phenyl position of lead compound **NK-189**[19] led to benzamides as noncharged alternatives. By further optimization of the cap moiety, several highly potent and selective DENV inhibitors were discovered. Those noncharged compounds are valuable and novel leads in the exploration of selective flaviviral inhibitors. The presented inhibitors show low micromolar to submicromolar activities in the DENV2proHeLa assay as well as in the DENV-2 titer reduction assay and lead to complete

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Table 5. Passive Membrane Permeability of Selected Compounds Determined by PAMPA

cpd.	M (g/mol)	C_{Acc} (μM)[a]	C_{Don} (μM)[b]	P_e (10^{-6} cm/s)[c]	R (%)[d]
caffeine	195	33 ± 4	173 ± 25	7.3	2.4
carbamazepine	236	42 ± 6	187 ± 4	8.9	−7.6
phenytoin	252	25 ± 2	166 ± 7	5.7	8.7
MB-53[16]	645		not permeable at a detectable range		
NK-189[19]	502	7.5 ± 0.7	171 ± 11	1.6	12
18	488		not permeable at a detectable range		
57	586	3.0 ± 1.4	180 ± 15	0.61	8.9
67	572	9.5 ± 0.9	164 ± 12	2.2	15
70	597	4.5 ± 1.0	177 ± 7	1.0	9.9
71	640	6.9 ± 1.0	188 ± 8	1.4	3.7
74	616	3.7 ± 1.3	180 ± 7	0.8	8.7
77	584	8.6 ± 1.0	178 ± 7	1.8	8.1
84	590	3.7 ± 0.7	187 ± 7	0.7	5.5
85	546	2.1 ± 0.2	169 ± 9	0.45	15
88	565	8.1 ± 1.7	181 ± 3	1.7	7.1

[a]Concentration of the compound in the acceptor plate after 5 h of incubation. [b]Concentration of the compound in the donor plate after 5 h of incubation. [c]Permeability of the compound calculated according to the literature.[50] [d]Mass retention of the compound calculated according to the literature.[50]

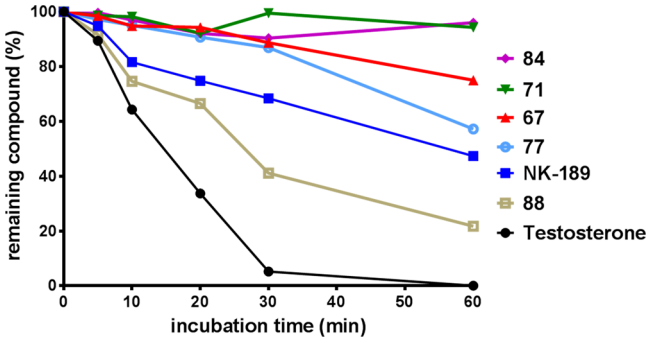

Figure 4. Degradation of compounds and reference substance testosterone in the presence of rat liver microsomes.

inhibition of viral replication at a concentration of 12.5 μM. Cytotoxic activity and interference with off-target serine proteases are absent from most of the compounds, resulting in excellent selectivity indices. Most of the compounds are capable of permeating through phospholipid membranes and have remarkable stability against rat liver microsomes as well as against the pancreatic enzymes α-chymotrypsin and trypsin.

The present study also indicates a need to further investigate the weak or absent correlation of biochemical assay results that use isolated flaviviral proteases versus the cellular antiviral activities, which has likely contributed to the failure of antiflaviviral drug discovery in the past. A reconsideration of screening procedures (e.g., addition of nonionic detergents) and optimization aims may be required in the field of flaviviral proteases. Since other working groups reported similar observations,[38−41] future studies will be directed on the investigation of this issue.

EXPERIMENTAL SECTION

Reagents and Solvents. All chemicals for the synthesis of precursors were obtained from Sigma-Aldrich (Germany), Alfa Aesar (Germany), Thermo Fisher Scientific (Germany/United States), Acros Organics (Belgium), TCI Europe (Belgium), and Carbolution Chemicals (Germany) and were of analytical grade. The amino acids were purchased from Carbolution Chemicals (Germany), Alfa Aesar (Germany), and TCI Europe (Belgium) or synthesized according to procedures described. COMU, HATU, and 2-chlorotrityl chloride resin (200−400 mesh; loading capacity, 1.6 mmol Cl^-/g resin; cross-linked with 1% DVB) were purchased from Carbolution Chemicals (Germany). Fmoc-Rink amide resin (75−150 mesh; loading capacity, 0.68 mmol/g resin) was purchased from Iris Biotech (Germany). Solvents were used as obtained from the commercial suppliers.

Equipment and Analytical Methods. The progress of the reactions was determined by thin-layer chromatography (TLC) on Merck silica gel plates 60 F_{254} (UV detection). Flash chromatography was performed on a Biotage Isolera One purification system using silica gel (0.060−0.200 mm) cartridges (KP-Sil) and UV monitoring at 254 and 280 nm. NMR spectra were recorded on a Varian NMR instrument at 300 or 500 MHz, 300 K; chemical shifts (δ) are given in parts per million (ppm). Residual peaks of nondeuterated solvents were used as an internal standard: $CDCl_3$ (δ ppm, 7.26), CD_3OD (δ ppm, 3.31), DMSO-d_6 (δ ppm, 2.50), CD_3CN (δ ppm, 1.94), and acetone-d_6 (δ ppm, 2.05). Coupling constants (J) are given in hertz (Hz). Multiplicity is reported as s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), dd (doublet of doublet), m (multiplet), and br (broad). Mass spectra (HR-ESI) of all compounds were measured on a Bruker micrOTOF-Q II instrument. Analysis of the compounds and intermediates was carried out using solutions in water, methanol, acetonitrile, or water/methanol. Purity of inhibitors was determined by HPLC on a Jasco HPLC system with a Jasco UV-2070 Plus Intelligent UV/VIS detector on an RP-18 column (ReproSil-Pur-ODS-3, Dr. Maisch GmbH, Germany; 5 μm, 50 mm × 2 mm). Purity for the final compounds is ≥95% unless indicated otherwise (see Table S4).

*4-(Benzyloxy)benzoic Acid (**89**).* To a suspension of 4-hydroxybenzoic acid (499 mg, 3.6 mmol) and K_2CO_3 (2501 mg, 18.1 mmol) in DMF was added respective benzyl bromide (0.903 mL, 7.6 mmol). The solution was warmed to 80 °C and was stirred for 2−3 days. The reaction mixture was diluted with ethyl acetate and washed with 1 N HCl, saturated $NaHCO_3$ solution, and water. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to give an oil. This was dissolved in a mixture of MeOH and 2 N aqueous NaOH (1:1) and was stirred at 50 °C for 2 h. After the pH was adjusted to 2 with concentrated HCl solution, the resulting precipitate was collected by filtration and dried under reduced pressure (771 mg, 93% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.64 (s, 1H), 7.91 (d, J = 8.7 Hz, 2H), 7.53−7.29 (m, 5H), 7.09 (d, J = 8.7 Hz, 2H), 5.17 (s, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 167.0, 161.9, 136.5, 131.4, 128.5, 127.8, 123.2, 114.6, 69.5. HRMS (ESI): m/z $[M-H]^-$ calcd for $C_{14}H_{11}O_3$, 227.0714; found, 227.0725.

*4-(Isopentyloxy)benzoic Acid (**90**).* 1-Bromo-3-methylbutane (2.290 mL, 19.1 mmol), 4-hydroxybenzoic acid (1202 mg, 8.7 mmol), and K_2CO_3 (2644 mg, 19.1 mmol) were mixed in DMF (25

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mL) and stirred at 100 °C overnight. The reaction mixture was poured into ice water (25 mL) and was extracted 3 times with ethyl acetate. The combined organic layers were washed with saturated sodium bicarbonate solution, water, and brine and dried over anhydrous $MgSO_4$. After concentration of the product under reduced pressure, the resulting residue was suspended in 2 N NaOH (25 mL) and was stirred at 60 °C overnight. The mixture was acidified to pH 1−2, and the resulting precipitate was collected by filtration to yield a white solid (1006 mg, 56% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.25 (s, 1H), 7.87 (d, J = 8.5 Hz, 2H), 6.98 (d, J = 8.5 Hz, 2H), 4.00 (t, J = 6.4 Hz, 2H), 1.78−1.65 (m, 1H), 1.57−1.47 (m, 2H), 0.90 (s, 6H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 165.3, 162.5, 131.1, 122.0, 114.3, 66.2, 37.2, 24.7, 22.3. HRMS (ESI): m/z [M−H]$^-$ calcd for $C_{12}H_{15}O_3$, 207.1027; found, 207.1005.

*Methyl 4-Hydroxybenzoate (**91**).* To a solution of 4-hydroxybenzoic acid (2505 mg, 18.1 mmol) in dry MeOH (20 mL) was added $SOCl_2$ (3.944 mL, 54.3 mmol) dropwise at 0 °C. The mixture was heated to reflux and was stirred for 24 h. The solution was concentrated in vacuo to afford a white solid (2740 mg, 99% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.30 (s, 1H), 7.81 (d, J = 8.6 Hz, 2H), 6.84 (d, J = 8.6 Hz, 2H), 3.77 (s, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 166.1, 162.0, 131.4, 120.3, 115.3, 51.6. HRMS (ESI): m/z [M−H]$^-$ calcd for $C_8H_7O_3$, 151.0401; found, 151.0379.

*4-(Methoxycarbonyl)phenylboronic Acid (**92**).* To a solution of 4-boronobenzoic acid (554 mg, 3,3 mmol) in dry MeOH (15 mL) was added $SOCl_2$ (0.727 mL, 10.0 mmol) dropwise at 0 °C. The mixture was heated to 50 °C and was stirred for 5 h. The solution was concentrated in vacuo to afford a white solid (590 mg, 98% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.18 (s, 2H), 8.00 (d, J = 5.8 Hz, 2H), 7.90 (d, J = 5.4 Hz, 2H), 3.85 (s, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 166.5, 134.1, 130.9, 128.0, 52.1. HRMS (ESI): m/z [M−H]$^-$ calcd for $C_8H_8BO_4$, 179.0518; found, 179.0523.

General Procedure for the Synthesis of 4-Phenyloxybenzoic Acid Derivatives (Procedure A). Methyl 4-phenoxybenzoate derivatives were synthesized according to a procedure modified from the literature.[23] Molecular sieves (4 Å) were given to a mixture of **91** (1 equiv), respective arylboronic acid (2 equiv), and $Cu(OAc)_2$ (2 equiv). DCM (10−15 mL) was added, and the resulting colored suspension was treated with 4 equiv of pyridine. After stirring the reaction mixture at an ambient atmosphere overnight, the suspension was dried under reduced pressure. The resulting residue was suspended in H_2O and ethyl acetate. The suspension was filtered, and the filtrate was washed 2 times with 1 N HCl, 0.2 N NaOH, and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo.

The methyl ester of carboxylic acid was cleaved by dissolving the compound in a solution of LiOH (2−3 equiv) in THF/H_2O (2:1, 10 mL). The solution was stirred for 3 h. The mixture was adjusted to pH 2 with concentrated HCl, and a precipitate was formed. The precipitate was filtered, washed with several portions of H_2O, and dried under reduced pressure. The crude mixture was purified by flash chromatography if necessary or used for subsequent synthetic steps without further purification.

*4-(2-Methoxyphenoxy)benzoic Acid (**93**).* Following the general procedure, **91** (228 mg, 1.5 mmol), 2-methoxyphenylboronic acid (455 mg, 3.0 mmol), $Cu(OAc)_2$ (544 mg, 3.0 mmol), and pyridine (0.484 mL, 6.0 mmol) were reacted together to give the respective methyl ester (**103**) as a yellow oil (165 mg, 43% yield). The deprotection of carboxylic acid was performed as described in the general procedure. In short, **103** (161 mg, 0.6 mmol) and a solution of LiOH (39 mg, 1.6 mmol) were reacted together to afford **93** as a pale yellow solid (141 mg, 93% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.69 (s, 1H), 7.89 (d, J = 8.9 Hz, 2H), 7.21 (t, J = 8.6 Hz, 1H), 7.12 (t, J = 8.5 Hz, 1H), 7.02 (d, J = 8.9 Hz, 1H), 6.87 (d, J = 8.9 Hz, 2H), 6.72 (d, J = 8.8 Hz, 1H), 3.79 (s, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 167.3, 162.1, 151.5, 142.2, 131.5, 123.2, 122.6, 121.5, 119.3, 115.5, 113.9, 55.8. HRMS (ESI): m/z [M−H]$^-$ calcd for $C_{14}H_{11}O_4$, 243.0663; found, 243.0676.

*4-(4-Cyanophenoxy)benzoic Acid (**94**).* Following the general procedure, **91** (255 mg, 1.7 mmol), 4-cyanophenylboronic acid (493 mg, 3.4 mmol), $Cu(OAc)_2$ (609 mg, 3.4 mmol), and pyridine (0.541 mL, 6.7 mmol) were reacted together to give the respective methyl ester (**104**) as a yellow oil (285 mg, 67% yield). The deprotection of carboxylic acid was performed as described in the general procedure. In short, **104** (196 mg, 0.8 mmol) and a solution of LiOH (46 mg, 1.9 mmol) were reacted together to afford **94** as a white solid (169 mg, 91% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.80 (s, 1H), 8.01 (s, J = 8.5 Hz, 2H), 7.89 (d, J = 8.4 Hz, 2H), 7.23 (d, J = 8.9 Hz, 2H), 7.19 (d, J = 8.7 Hz, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 166.6, 159.7, 158.7, 134.8, 131.8, 127.0, 119.2, 118.6, 116.4, 106.3. HRMS (ESI): m/z [M−H]$^-$ calcd for $C_{14}H_8NO_3$, 238.0510; found, 238.0510.

*4-(4-(Trifluoromethyl)phenoxy)benzoic Acid (**95**).* Following the general procedure, **91** (101 mg, 0.7 mmol), 4-(trifluoromethyl)-phenylboronic acid (248 mg, 1.3 mmol), $Cu(OAc)_2$ (239 mg, 1.3 mmol), and pyridine (0.212 mL, 4.0 mmol) were reacted together to give the respective methyl ester (**105**) as a yellow oil (126 mg, 65% yield). The deprotection of carboxylic acid was performed as described in the general procedure. In short, **105** (90 mg, 0.3 mmol) and a solution of LiOH (18 mg, 0.8 mmol) were reacted together to afford **95** as a white solid (86 mg, 92% yield). ^{1}H NMR (300 MHz, acetone-d_6): δ 12.76 (s, 1H), 8.11 (d, J = 8.9 Hz, 2H), 7.79 (d, J = 8.6 Hz, 2H), 7.28 (d, J = 8.4 Hz, 2H), 7.18 (d, J = 8.8 Hz, 2H). ^{13}C NMR (APT, 75 MHz, acetone-d_6): δ 167.8, 163.1, 160.8, 132.9, 128.4, 128.4, 128.0, 120.3, 120.2, 119.6. HRMS (ESI): m/z [M−H]$^-$ calcd for $C_{14}H_8F_3O_3$, 281.0431; found, 281.0441.

*4-(Benzo[d][1,3]dioxol-5-yloxy)benzoic Acid (**96**).* Following the general procedure, **91** (181 mg, 1.2 mmol), benzo[d][1,3]dioxol-5-ylboronic acid (393 mg, 2.4 mmol), $Cu(OAc)_2$ (428 mg, 2.4 mmol), and pyridine (0.382 mL, 4.7 mmol) were reacted together to give the respective methyl ester (**106**) as a brown oil (108 mg, 34% yield). The deprotection of carboxylic acid was performed as described in the general procedure. In short, **106** (107 mg, 0.4 mmol) and a solution of LiOH (24 mg, 1.0 mmol) were reacted together to afford **96** as a brown solid (89 mg, 87% yield). ^{1}H NMR (300 MHz, acetone-d_6): δ 12.80 (s, 1H), 8.02 (d, J = 8.8 Hz, 2H), 7.00 (d, J = 8.6 Hz, 2H), 6.90 (d, J = 8.4 Hz, 1H), 6.80 (d, J = 8.4 Hz, 1H), 6.70 (s, 1H), 6.06 (s, 2H). ^{13}C NMR (APT, 75 MHz, acetone-d_6): δ 170.5, 163.5, 150.7, 150.6, 149.7, 132.3, 125.4, 117.3, 115.9, 113.8, 103.5, 101.8. HRMS (ESI): m/z [M−H]$^-$ calcd for $C_{14}H_9O_5$, 257.0455; found, 257.0443.

*4-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yloxy)benzoic Acid (**97**).* Following the general procedure, **91** (214 mg, 1.4 mmol), 2,3-dihydrobenzo[b][1,4]dioxin-6-ylboronic acid (506 mg, 2.8 mmol), $Cu(OAc)_2$ (511 mg, 2.8 mmol), and pyridine (0.454 mL, 5.6 mmol) were reacted together to give the respective methyl ester (**107**) as a brown oil (198 mg, 49% yield). The deprotection of carboxylic acid was performed as described in the general procedure. In short, **107** (157 mg, 0.5 mmol) and a solution of LiOH (33 mg, 1.4 mmol) were reacted together to afford **97** as a brown solid (137 mg, 92% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.69 (s, 1H), 7.93 (d, J = 8.6 Hz, 2H), 6.99 (d, J = 8.6 Hz, 2H), 6.92 (d, J = 8.7 Hz, 1H), 6.82 (d, J = 8.5 Hz, 1H), 6.67 (s, 1H), 4.25 (s, 4H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 165.7, 162.1, 151.0, 148.1, 144.1, 131.5, 123.5, 116.4, 113.2, 111.2, 107.3, 64.2, 64.2. HRMS (ESI): m/z [M−H]$^-$ calcd for $C_{15}H_{11}O_5$, 271.0612; found, 271.0610.

General Procedure for the Synthesis of 4-Phenyloxybenzoic Acid Derivatives (Procedure B). Methyl 4-phenoxybenzoate derivatives were synthesized according to a procedure modified from the literature.[23] Molecular sieves (4 Å) were given to a mixture of **92** (2 equiv), the respective phenol derivative (1 equiv), and $Cu(OAc)_2$ (2 equiv). DCM (15 mL) was added, and the resulting colored suspension was treated with 4 equiv of pyridine. After stirring the reaction mixture at an ambient atmosphere for two days, the suspension was dried under reduced pressure. The resulting residue was suspended in H_2O and ethyl acetate. The suspension was filtered, and the filtrate was washed 2 times with 1 N HCl, 0.2 N NaOH, and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo.

The methyl ester of carboxylic acid was cleaved by dissolving the compound in a solution of LiOH (3.5 equiv) in THF/H_2O (2:1, 10

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mL) at 0 °C. The solution was stirred at room temperature overnight. The mixture was adjusted to pH 2 with concentrated HCl, and a precipitate was formed. The precipitate was filtered, washed with several portions of H_2O, and dried under reduced pressure. The crude mixture was purified by flash chromatography if necessary or used for subsequent synthetic steps without further purification.

*4-(3-Oxo-2,3-dihydro-1H-inden-4-yloxy)benzoic Acid (**98**).* Following the general procedure B, **92** (270 mg, 1.5 mmol), 7-hydroxy-2,3-dihydro-1*H*-inden-1-one (111 mg, 0.8 mmol), $Cu(OAc)_2$ (273 mg, 1.5 mmol), and pyridine (0.242 mL, 3.0 mmol) were reacted together to give the respective methyl ester (**108**) as a brown oil (226 mg, 53% yield). The deprotection of carboxylic acid was performed as described in the general procedure. In short, **108** (198 mg, 0.7 mmol) and a solution of LiOH (59 mg, 2.5 mmol) were reacted together to afford **98** as a pale red solid (180 mg, 96% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.46 (s, 1H), 7.79 (d, J = 8.7 Hz, 2H), 7.43 (t, J = 7.8 Hz, 1H), 7.01 (d, J = 8.5 Hz, 1H), 6.82 (d, J = 8.7 Hz, 2H), 6.72 (d, J = 8.1 Hz, 1H), 3.15−2.96 (m, 2H), 2.64−2.52 (m, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 205.7, 167.2, 161.6, 156.6, 156.2, 134.1, 131.5, 128.1, 123.3, 121.4, 115.1, 113.7, 36.1, 25.2. HRMS (ESI): m/z [M−H]$^-$ calcd for $C_{16}H_{13}O_4$, 267.0663; found, 267.0699.

*4-(4-Chlorophenoxy)benzoic Acid (**99**).* Following the general procedure B, **92** (159 mg, 0.9 mmol), 4-chlorophenol (57 mg, 0.4 mmol), $Cu(OAc)_2$ (160 mg, 0.9 mmol), and pyridine (0.143 mL, 1.8 mmol) were reacted together to give the respective methyl ester (**109**) as a yellow oil (162 mg, 70% yield). The deprotection of carboxylic acid was performed as described in the general procedure. In short, **109** (152 mg, 0.6 mmol) and a solution of LiOH (49 mg, 2.0 mmol) were reacted together to afford **99** as a white solid (127 mg, 88% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.79 (s, 1H), 7.95 (d, J = 8.8 Hz, 2H), 7.49 (d, J = 8.9 Hz, 2H), 7.15 (d, J = 8.9 Hz, 2H), 7.06 (d, J = 8.8 Hz, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 166.7, 160.5, 154.1, 131.7, 130.2, 128.5, 125.7, 121.6, 117.5. HRMS (ESI): m/z [M−H]$^-$ calcd for $C_{13}H_{10}ClO_3$, 247.0167; found, 247.0169.

*4-(3-Chlorophenoxy)benzoic Acid (**100**).* Following the general procedure B, **92** (156 mg, 0.9 mmol), 3-chlorophenol (56 mg, 0.4 mmol), $Cu(OAc)_2$ (157 mg, 0.9 mmol), and pyridine (0.140 mL, 1.7 mmol) were reacted together to give the respective methyl ester (**110**) as a yellow oil (203 mg, 89% yield). The deprotection of carboxylic acid was performed as described in the general procedure. In short, **110** (193 mg, 0.7 mmol) and a solution of LiOH (62 mg, 2.6 mmol) were reacted together to afford **100** as a white solid (127 mg, 69% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.84 (s, 1H), 7.97 (d, J = 8.8 Hz, 2H), 7.46 (t, J = 8.1 Hz, 1H), 7.29 (d, J = 8.1 Hz, 1H), 7.22 (s, 1H), 7.13−7.05 (m, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 166.7, 160.1, 156.3, 134.2, 131.8, 131.7, 126.0, 124.5, 119.8, 118.4, 117.8. HRMS (ESI): m/z [M−H]$^-$ calcd for $C_{13}H_{10}ClO_3$, 247.0167; found, 247.0158.

General Procedure for Peptide Coupling in Solution. Respective carboxylic acid (1.0 equiv) and HATU (1.2 equiv) were suspended in DCM (2−10 mL). The mixture was cooled to 0 °C, and the respective amine (1.2 equiv) was added prior to the dropwise addition of TMP. DMF was added until all solids were dissolved, and the mixture was stirred at room temperature for about 2 h. The reaction was quenched with the addition of a solution of TFA in DCM (5%, 5 mL). All solvents were removed in vacuo, and the resulting residue was dissolved in ethyl acetate. The ethyl acetate phase was washed 2 times with 1 N HCl, 0.05 N NaOH, and water. The combined organic phase was dried over anhydrous $MgSO_4$, concentrated under reduced pressure, and purified by preparative HPLC. After purification, all organic solvents were evaporated, and the compounds were freeze-dried in H_2O/ACN.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-oxo-2-(piperidin-1-yl)ethyl)-hexanamide (**1**).* Compound **1** was synthesized according to the general procedure for peptide coupling in solution from **102** (27 mg, 0.08 mmol), HATU (33 mg, 0.09 mmol), piperidine·HCl (11 mg, 0.09 mmol), and TMP (0.023 mL, 0.17 mmol) and was obtained as a yellow oil (18 mg, 56% yield). ^{1}H NMR (300 MHz, acetone-d_6): δ 7.54−7.43 (m, 3H), 7.43−7.27 (m, 5H), 6.98 (d, J = 8.7 Hz, 2H), 5.86−5.78 (m, 1H), 5.11 (s, 2H), 3.47−3.27 (m, 4 H), 2.26−2.10 (m, 2H), 1.64−1.13 (m, 12H), 0.85 (t, J = 6.8 Hz, 3H). ^{13}C NMR (APT, 126 MHz, DMSO-d_6): δ 171.4, 167.9, 157.7, 137.0, 130.4, 129.1, 128.4, 127.8, 127.6, 114.7, 69.2, 52.2, 45.8, 42.6, 34.8, 30.8, 25.5, 25.2, 24.9, 23.8, 21.8, 13.8. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{26}H_{35}N_2O_3$, 423.2642; found, 423.2658.

*(S)-N-(2-(Benzylamino)-1-(4-(benzyloxy)phenyl)-2-oxoethyl)-hexanamide (**2**).* Compound **2** was synthesized according to the general procedure for peptide coupling in solution from **102** (21 mg, 0.06 mmol), HATU (27 mg, 0.07 mmol), benzylamine (0.008 mL, 0.07 mmol), and TMP (0.010 mL, 0.08 mmol) and was obtained as a white powder (14 mg, 54% yield). ^{1}H NMR (300 MHz, acetone-d_6): δ 7.83 (t, J = 5.9 Hz, 1H), 7.53 (d, J = 7.6 Hz, 1H), 7.47 (d, J = 6.7 Hz, 2H), 7.43−7.29 (m, 5H), 7.29−7.15 (m, 5H), 6.96 (d, J = 8.7 Hz, 2H), 5.51 (d, J = 7.7 Hz, 1H), 5.12 (s, 2H), 4.39 (d, J = 6.2 Hz, 2H), 2.25 (t, J = 7.5 Hz, 2H), 1.66−1.52 (m, 2H), 1.36−1.21 (m, 4H), 0.86 (t, J = 6.8 Hz, 3H). ^{13}C NMR (APT, 126 MHz, DMSO-d_6): δ 171.9, 170.3, 157.7, 139.1, 137.1, 131.2, 128.4, 128.2, 127.8, 127.6, 127.0, 126.7, 114.5, 69.1, 55.6, 42.0, 34.9, 30.9, 24.9, 21.9, 13.9. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{28}H_{33}N_2O_3$, 445.2486; found, 445.2487.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-oxo-2-(phenethylamino)ethyl)-hexanamide (**3**).* Compound **3** was synthesized according to the general procedure for peptide coupling in solution from **102** (27 mg, 0.08 mmol), HATU (34 mg, 0.09 mmol), phenylethylamine·HCl (14 mg, 0.09 mmol), and TMP (0.023 mL, 0.17 mmol) and was obtained as a white powder (19 mg, 56% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.30 (d, J = 8.1 Hz, 1H), 8.23 (t, J = 5.3 Hz, 1H), 7.47−7.31 (m, 5H), 7.27−7.15 (m, 5H), 7.09 (d, J = 6.6 Hz, 2H), 6.94 (d, J = 8.6 Hz, 2H), 5.35 (d, J = 8.1 Hz, 1H), 5.10 (s, 2H), 3.28−3.18 (m, 2H), 2.66 (t, J = 7.1 Hz, 2H), 2.16 (t, J = 7.4 Hz, 2H), 1.46 (p, J = 7.2 Hz, 2H), 1.33−1.14 (m, 4H), 0.84 (t, J = 6.9 Hz, 3H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{29}H_{35}N_2O_3$, 459.2642; found, 459.2641.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(4-chlorobenzylamino)-2-oxoethyl)hexanamide (**4**).* Compound **4** was synthesized according to the general procedure for peptide coupling in solution from **102** (21 mg, 0.06 mmol), HATU (27 mg, 0.07 mmol), 4-chlorobenzylamine (0.009 mL, 0.07 mmol), and TMP (0.010 mL, 0.08 mmol) and was obtained as a white powder (15 mg, 54% yield). ^{1}H NMR (300 MHz, acetone-d_6): δ 7.97 (t, J = 5.4 Hz, 1H), 7.59 (d, J = 7.6 Hz, 1H), 7.47 (d, J = 7.1 Hz, 2H), 7.43−7.31 (m, 5H), 7.30−7.17 (m, 4H), 6.97 (d, J = 8.7 Hz, 2H), 5.49 (d, J = 7.4 Hz, 1H), 5.12 (s, 2H), 4.37 (d, J = 6.1 Hz, 2H), 2.25 (t, J = 7.5 Hz, 2H), 1.64−1.51 (m, 2H), 1.35−1.20 (m, 4H), 0.85 (t, J = 6.9 Hz, 3H). ^{13}C NMR (APT, 126 MHz, DMSO-d_6): δ 171.9, 170.4, 157.7, 138.3, 137.1, 131.2, 131.0, 128.8, 128.4, 128.1, 127.8, 127.6, 114.5, 69.1, 55.7, 41.3, 34.8, 30.9, 24.9, 21.9, 13.9. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{28}H_{32}ClN_2O_3$, 479.2096; found, 479.2081.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(4-methoxyphenylamino)-2-oxoethyl)hexanamide (**5**).* Compound **5** was synthesized according to the general procedure for peptide coupling in solution from **102** (28 mg, 0.08 mmol), HATU (35 mg, 0.09 mmol), 4-methoxyaniline (0.011 mL, 0.09 mmol), and TMP (0.018 mL, 0.14 mmol) and was obtained as a white powder (33 mg, 92% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.12 (s, 1H), 8.47 (d, J = 7.9 Hz, 1H), 7.48 (d, J = 9.0 Hz, 2H), 7.44−7.30 (m, 7H), 6.99 (d, J = 8.6 Hz, 2H), 6.86 (d, J = 8.8 Hz, 2H), 5.54 (d, J = 7.9 Hz, 1H), 5.09 (s, 2H), 3.70 (s, 3H), 2.20 (t, J = 7.4 Hz, 2H), 1.49 (p, J = 7.2 Hz, 2H), 1.34−1.15 (m, 4H), 0.84 (t, J = 6.7 Hz, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 172.0, 168.6, 157.8, 155.3, 137.0, 132.0, 130.7, 128.44, 128.41, 127.8, 127.6, 120.6, 114.7, 113.9, 69.2, 56.1, 55.1, 34.8, 30.9, 24.9, 21.9, 13.9. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{28}H_{33}N_2O_4$, 461.2435; found, 461.2431.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(4-methoxybenzylamino)-2-oxoethyl)hexanamide (**6**).* Compound **6** was synthesized according to the general procedure for peptide coupling in solution from **102** (21 mg, 0.06 mmol), HATU (27 mg, 0.07 mmol), 4-methoxybenzylamine (0.009 mL, 0.07 mmol), and TMP (0.010 mL, 0.08 mmol) and was obtained as a white powder (18 mg, 64% yield). ^{1}H NMR (300 MHz, acetone-d_6): δ 7.74 (t, J = 6.0 Hz, 1H), 7.57−7.44 (m, 3H),

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7.44−7.28 (m, 5H), 7.13 (d, J = 8.6 Hz, 2H), 6.96 (d, J = 8.7 Hz, 2H), 6.81 (d, J = 8.6 Hz, 2H), 5.49 (d, J = 7.6 Hz, 1H), 5.11 (s, 2H), 4.31 (d, J = 5.8 Hz, 2H), 3.75 (s, 3H), 2.25 (t, J = 7.5 Hz, 2H), 1.63−1.51 (m, 2H), 1.34−1.21 (m, 4H), 0.86 (t, J = 6.8 Hz, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 171.8, 170.1, 158.2, 157.7, 137.1, 131.3, 131.0, 128.8, 128.4, 127.8, 127.6, 126.7, 114.5, 113.6, 69.2, 55.6, 55.0, 41.5, 34.9, 30.9, 25.0, 21.9, 13.9. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{29}H_{35}N_2O_4$, 497.2411; found, 497.2409.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-methoxybenzylamino)-2-oxoethyl)hexanamide (**7**).* Compound **7** was synthesized according to the general procedure for peptide coupling in solution from **102** (28 mg, 0.08 mmol), HATU (35 mg, 0.09 mmol), (3-methoxyphenyl)methanamine (0.012 mL, 0.09 mmol), and TMP (0.018 mL, 0.14 mmol) and was obtained as a white powder (24 mg, 65% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.66 (t, J = 6.0 Hz, 1H), 8.37 (d, J = 7.9 Hz, 1H), 7.48−7.28 (m, 7H), 7.15 (t, J = 7.8 Hz, 1H), 6.97 (d, J = 8.7 Hz, 2H), 6.80−6.68 (m, 3H), 5.43 (d, J = 7.9 Hz, 1H), 5.09 (s, 2H), 4.24 (d, J = 5.8 Hz, 2H), 3.65 (s, 3H), 2.18 (t, J = 7.4 Hz, 2H), 1.47 (p, J = 7.2 Hz, 2H), 1.32−1.13 (m, 4H), 0.83 (t, J = 6.9 Hz, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 171.9, 170.4, 159.3, 157.7, 140.8, 137.1, 131.2, 129.2, 128.4, 127.8, 127.6, 119.1, 114.5, 112.6, 112.0, 69.2, 55.7, 54.8, 41.9, 34.9, 30.9, 24.9, 21.9, 13.9. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{29}H_{35}N_2O_4$, 475.2591; found, 475.2606.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(4-(benzyloxy)phenylamino)-2-oxoethyl)hexanamide (**8**).* Compound **8** was synthesized according to the general procedure for peptide coupling in solution from **102** (28 mg, 0.08 mmol), HATU (35 mg, 0.09 mmol), **111** (22 mg, 0.09 mmol), and TMP (0.013 mL, 0.10 mmol) and was obtained as a white powder (30 mg, 71% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.47−7.28 (m, 14H), 6.98−6.85 (m, 4H), 5.64 (s, 1H), 5.03 (s, 2H), 5.02 (s, 2H), 2.25 (t, J = 7.6 Hz, 2H), 1.68−1.56 (m, 2H), 1.33−1.21 (m, 4H), 0.86 (t, J = 6.6 Hz, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 172.0, 168.7, 157.8, 154.3, 137.1, 137.0, 132.2, 130.7, 128.44, 128.41, 128.37, 127.78, 127.75, 127.64, 127.57, 120.5, 114.9, 114.7, 69.3, 69.2, 56.1, 34.8, 30.9, 24.9, 21.9, 13.9. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{34}H_{37}N_2O_4$, 537.2748; found, 537.2752.

*(S)-1-(2-(4-(Benzyloxy)phenyl)-2-hexanamidoacetyl)-N-phenylpiperidine-4-carboxamide (**12**).* Compound **12** was synthesized according to the general procedure for peptide coupling in solution from **102** (28 mg, 0.08 mmol), HATU (35 mg, 0.09 mmol), **112** (19 mg, 0.09 mmol), and TMP (0.013 mL, 0.10 mmol) and was obtained as a white powder (27 mg, 64% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.55−7.28 (m, 9H), 7.16−6.89 (m, 5H), 5.83 (s, 1H), 5.03 (s, 2H), 3.56−3.28 (m, 4H), 2.73−2.60 (m, 1H), 2.30−1.93 (m, 6H), 1.71−1.53 (m, 2H), 1.37−1.19 (m, 4H), 0.85 (t, J = 6.9 Hz, 3H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{33}H_{40}N_3O_4$, 542.3013; found, 542.3000.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-oxo-2-(4-sulfamoylphenylamino)ethyl)hexanamide (**26**).* Compound **26** was synthesized according to the general procedure for peptide coupling in solution from **102** (20 mg, 0.06 mmol), HATU (26 mg, 0.07 mmol), **113** (12 mg, 0.07 mmol), and TMP (0.010 mL, 0.07 mmol) and was obtained as a pale yellow solid (2 mg, 7% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.19 (s, 1H), 8.49 (d, J = 7.8 Hz, 1H), 7.89−7.47 (m, 4H), 7.47−7.25 (m, 7H), 7.00 (d, J = 8.7 Hz, 2H), 5.53 (d, J = 7.8 Hz, 1H), 5.10 (s, 2H), 2.21 (t, J = 7.3 Hz, 2H), 1.57−1.26 (m, 2H), 1.36−1.13 (m, 4H), 0.84 (t, J = 6.8 Hz, 3H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{27}H_{32}N_3O_5S$, 510.2057; found, 510.2069.

*(S)-N-(2-(4-Acetamidobenzylamino)-1-(4-(benzyloxy)phenyl)-2-oxoethyl)hexanamide (**21**).* **101** (31 mg, 0.05 mmol) was dissolved in DCM (5 mL) and cooled to 0 °C with an ice-water bath. Triethylamine (0.009 mL, 0.07 mmol) was added to the solution prior to the addition of acetyl chloride (0.004 mL, 0.06 mmol). The mixture was warmed to room temperature and stirred overnight before the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate and washed 2 times with saturated sodium bicarbonate solution and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified with reversed-phase liquid chromatography and freeze-dried in H_2O/ACN to afford a white powder (16 mg, 59% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.87 (s, 1H), 8.61 (t, J = 5.7 Hz, 1H), 8.35 (d, J = 8.0 Hz, 1H), 7.48−7.28 (m, 9H), 7.06 (d, J = 8.4 Hz, 2H), 6.96 (d, J = 8.6 Hz, 2H), 5.41 (d, J = 8.0 Hz, 1H), 5.09 (s, 2H), 4.27−4.10 (m, 2H), 2.17 (t, J = 7.3 Hz, 2H), 2.01 (s, 3H), 1.47 (p, J = 7.3 Hz, 2H), 1.30−1.14 (m, 4H), 0.83 (t, J = 6.9 Hz, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 171.9, 170.2, 168.1, 157.7, 138.0, 137.1, 133.6, 131.2, 128.42, 128.39, 127.8, 127.6, 127.4, 118.8, 114.5, 69.2, 55.6, 41.6, 34.9, 30.9, 25.0, 24.0, 21.9, 13.9. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{30}H_{36}N_3O_4$, 502.2700; found, 502.2691.

*(S)-4-((2-(4-(Benzyloxy)phenyl)-2-hexanamidoacetamido)-methyl)-N-hydroxybenzamide (**24**).* Compound **24** was synthesized according to a procedure modified from the literature.[59] **25** (28 mg, 0.06 mmol) was dissolved in dry THF (1 mL) under a nitrogen atmosphere. To this solution, carbonyldiimidazole (14 mg, 0.09 mmol) in dry THF (0.5 mL) was added. The mixture was stirred for 1 h at room temperature, and hydroxylamine hydrochloride (8 mg, 0.11 mmol) in dry THF (1 mL) was added. The mixture was further stirred overnight, and the mixture was diluted with 5% aqueous $KHSO_4$. The resulting suspension was extracted with ethyl acetate and was washed 2 times with brine and water. The organic phase was concentrated in vacuo, filtered over anhydrous $MgSO_4$, and was further purified with reversed-phase liquid chromatography. The purified product was redissolved in water and freeze-dried to yield a white powder (6 mg, 21% yield). ^{1}H NMR (300 MHz, acetone-d_6): δ 10.68 (br s, 1H), 7.97−7.87 (m, 2H), 7.72 (d, J = 8.5 Hz, 2H), 7.57−7.51 (m, 1H), 7.48 (d, J = 7.5 Hz, 2H), 7.43−7.25 (m, 7H), 6.98 (d, J = 6.9 Hz, 2H), 5.53−5.47 (m, 1H), 5.13 (s, 2H), 4.50−4.41 (m, 2H), 2.26 (t, J = 7.7 Hz, 2H), 1.64−1.51 (m, 2H), 1.35−1.24 (m, 4H), 0.86 (t, J = 6.9 Hz, 3H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{29}H_{33}N_3NaO_5$, 526.2312; found, 526.2316.

*(S)-4-((2-(4-(Benzyloxy)phenyl)-2-(hexylamino)acetamido)-methyl)benzamide (**46**).* The peptide synthesis for **46** was done according to the general procedure for the synthesis of peptides on Rink amide resin. After drying the resin in vacuo (with compound **40** attached), a reductive amination was performed on resin. Therefore, the resin (130 mg) was reswollen in DMF for 30 min, hexanal was added (0.024 mL), and the resin was shaken for 3 h. The resin was washed with DMF, DCM, and dry MeOH. $NaBH_3CN$ (10 mg) in 1 mL of dry MeOH was added to the resin and was shaken overnight. The resin was washed with MeOH, DMF, DCM, and diethyl ether. It was dried under reduced pressure overnight, and the peptide was cleaved off the resin and purified by the standard procedure. ^{1}H NMR (300 MHz, acetone-d_6): δ 8.70 (t, J = 5.8 Hz, 1H), 7.81 (d, J = 8.1 Hz, 2H), 7.59 (d, J = 8.7 Hz, 2H), 7.48 (d, J = 7.0 Hz, 2H), 7.44−7.30 (m, 3H), 7.26 (d, J = 8.1 Hz, 2H), 7.04 (d, J = 8.7 Hz, 2H), 6.83 (br s, 2H), 5.39 (s, 1H), 5.13 (s, 2H), 4.58−4.35 (m, 2H), 3.11−2.83 (m, 2H), 1.84−1.67 (m, 2H), 1.33−1.11 (m, 6H), 0.82 (t, J = 6.7 Hz, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 167.5, 167.0, 159.3, 141.7, 136.8, 133.0, 130.1, 128.5, 127.9, 127.7, 127.5, 126.7, 124.1, 115.2, 69.3, 61.9, 45.4, 42.0, 30.6, 25.6, 25.1, 21.8, 13.8. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{29}H_{36}N_3O_3$, 474.2751; found, 474.2761.

*(S)-4-((2-(Benzylamino)-2-(4-(benzyloxy)phenyl)acetamido)-methyl)benzamide (**53**).* The peptide synthesis for **53** was done according to the general procedure for the synthesis of peptides on Rink amide resin. After drying the resin in vacuo (with compound **40** attached), a reductive amination was performed on resin. Therefore, the resin (130 mg) was reswollen in DMF for 30 min, benzaldehyde was added (0.026 mL), and the resin was shaken for 3 h. The resin was washed with DMF, DCM, and dry MeOH. $NaBH_3CN$ (10 mg) in 1 mL of dry MeOH was added to the resin and was shaken overnight. The resin was washed with MeOH, DMF, DCM, and diethyl ether. It was dried under reduced pressure overnight, and the peptide was cleaved off the resin and purified by the standard procedure. ^{1}H NMR (300 MHz, acetone-d_6): δ 8.58 (t, J = 5.7 Hz, 1H), 7.80 (d, J = 7.8 Hz, 2H), 7.59 (d, J = 8.3 Hz, 2H), 7.52−7.30 (m, 10H), 7.25 (d, J = 7.8 Hz, 2H), 7.06 (d, J = 7.7 Hz, 2H), 6.79 (br s, 2H), 5.33 (s, 1H), 5.14 (s, 2H), 4.56−4.36 (m, 2H), 4.35−4.15 (m, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 167.5, 166.9, 159.2, 141.6, 136.8,

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133.0, 130.2, 128.6, 128.5, 127.9, 127.7, 127.5, 126.8, 115.2, 69.3, 61.4, 48.9, 42.0. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{30}H_{30}N_3O_3$, 480.2282; found, 480.2280.

*(S)-4-((2-(4-(Benzyloxy)phenyl)-2-(heptylsulfonamido)-acetamido)methyl)benzamide (**45**).* Sodium heptanesulfonate (50 mg, 0.28 mmol) was refluxed in thionyl chloride (0.5 mL) under a nitrogen atmosphere. After 6 h, the mixture was cooled to room temperature, the volatiles were removed under reduced pressure, and the resulting yellow oil was dissolved in dry DCM under a nitrogen atmosphere. The solution was cooled with an ice-water bath, and NEt_3 (0.058 mL, 0.42 mmol) and **40** (110 mg, 0.28 mmol) were added slowly as a suspension in dry DCM. The mixture was allowed to warm to room temperature and was stirred for 16 h. DCM (10 mL) was added, and the mixture was washed with 1 N HCl, saturated $NaHCO_3$ solution, and water. The organic phase was dried over anhydrous $MgSO_4$ and concentrated in vacuo to yield an orange oil. The product was further purified with RP-HPLC, redissolved in H_2O/ACN, and freeze-dried to yield a yellow solid (3 mg, 21% yield). ^{1}H NMR (300 MHz, MeOH-d_4/D_2O): δ 7.77 (d, J = 8.5 Hz, 2H), 7.45−7.32 (m, 7H), 7.28 (d, J = 8.2 Hz, 2H), 7.02 (d, J = 8.0 Hz, 2H), 5.35 (s, 1H), 5.12 (s, 2H), 4.43 (s, 2H), 3.24−3.15 (m, 2H), 1.72−1.50 (m, 2H), 1.40−1.13 (m, 8H), 0.86 (t, J = 6.9 Hz, 3H). HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{30}H_{38}N_3O_5S$, 552.2527; found, 552.2513.

General Procedure for the Synthesis of Inhibitors and Intermediates on a Solid Support. All sequences and intermediates were assembled by stepwise solid-phase synthesis on 2-chlorotrityl chloride resin or Rink amid resin using the standard Fmoc strategy, as previously described.[17,42,60] Solid-phase synthesis was done manually in plastic syringes equipped with a frit; all steps were performed at room temperature under continuous shaking. The Rink amide resin was preswollen in DCM for at least 20 min and then washed 3× with DMF. For Fmoc deprotection, a piperidine solution (10−20% in DMF) was added 2× for 10 and 5 min. Following each deprotection or coupling step, the resin was washed 3× with DMF, 3× with DCM, and again 3× with DMF. COMU and TMP were used for coupling steps. In detail, the coupling solution contained the *Nα*-Fmoc-protected amino acid or the respective building block (2.0−3.0 equiv), COMU (2.0−3.0 equiv), and TMP (2.3−3.9 equiv) in DMF (1.0 mL per 100 mg of resin). The solution was added to the resin, and the mixture was shaken for 60−120 min. Afterward, the resin was washed as described before. Fmoc deprotection and coupling steps were iteratively repeated until the desired sequence was obtained. For the sequence containing 4-(OH)-phenylglycine, a final treatment with piperidine solution (20% in DMF) for 30 min was carried out after coupling of the cap to cleave any formed esters at the unprotected hydroxyl group. The resin loaded with the finished peptide or peptide hybrid was washed 5× with diethyl ether and dried under reduced pressure. The final product was cleaved 2× off the resin with TFA/TIPS/H_2O solution (95:1:4, 1−2 mL per 100 mg of resin) or TFA/H_2O/DCM solution (95:2.5:2.5, 1−2 mL per 100 mg of resin), and the mixture was shaken for 2 h. The cleavage solution was dispensed into cold diethyl ether or hexane (35 mL per 100 mg of resin), and the resulting precipitate was centrifuged (4000*g*, 10 min), washed with diethyl ether/hexane, and dried under reduced pressure. If the peptides were soluble in the organic solvent, then the organic phase was washed 2 times with a small amount of water, and all solvents were removed in vacuo. The resulting residue was coevaporated with toluene several times.

The peptide synthesis with 2-chlorotrityl chloride resin was performed as described with Rink amid resin with the exception of the loading and cleavage step. The respective Fmoc-protected first building block (1.5−3.0 equiv) was added to the resin in the syringe, and 1 mL of DMF and TMP (3 equiv) was added. If a hydroxyl functional group was coupled in the loading step, then 1 mL of pyridine (per 100 mg resin) was added instead of the DMF/TMP mixture. The resin was shaken overnight and capped before the Fmoc deprotection step. Therefore, the resin was washed with 3× DMF, 3× DCM, and again 3× DMF, and the capping solution (DCM/MeOH/TMP, 80:15:5 (v/v), 1 mL per 100 mg of resin) was added 2× for 20 min. The resin was washed with 3× DMF, 3× DCM, and again 3× DMF and was handled like the Rink amide resin in the next coupling steps. The resin loaded with the finished sequence was washed 5× with diethyl ether and dried under reduced pressure. The final product was cleaved off the resin 3 times with TFA/DCM solution (5:95, 1−2 mL per 100 mg of resin), and the mixture was shaken each time for at least 30 min. The cleavage solution was dispensed into a round-bottom flask, washed two times with a small amount of water, dried under reduced pressure, and coevaporated with toluene several times.

All final compounds were purified by preparative RP-HPLC on an ÄKTA Purifier, GE Healthcare (Germany), with an RP-18 pre and main column (Rephospher, Dr. Maisch GmbH, Germany; C18-DE, 5 μm, 30 mm × 16 mm, and 120 mm × 16 mm). The following conditions were used: eluent A, water (0.1% TFA); eluent B, methanol (0.1% TFA) or eluent A, water (0.1% TFA); eluent B, acetonitrile (0.1% TFA); flow rate, 8 mL/min; and gradient, 10% B (2.5 min), 100% B (23.5 min), 100% B (26 min), 10% B (26.1 min), and 10% B (30 min). Detection was performed at 214, 254, and 280 nm. After purification, the organic solvent was evaporated, and the compounds and intermediates were freeze-dried in H_2O/ACN and stored at −20 °C.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(4-(hydroxymethyl)-phenylamino)-2-oxoethyl)hexanamide (**9**).* ^{1}H NMR (300 MHz, CD_3OD/D_2O): δ 7.50 (d, J = 8.4 Hz, 2H), 7.44−7.26 (m, 9H), 7.00 (d, J = 8.6 Hz, 2H), 5.51 (s, 1H), 5.09 (s, 2H), 4.54 (s, 2H), 2.29 (t, J = 7.5 Hz, 2H), 1.68−1.53 (m, 2H), 1.36−1.25 (m, 4H), 0.88 (t, J = 6.9 Hz, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 172.1, 169.0, 157.9, 137.6, 137.4, 137.0, 130.5, 128.5, 128.4, 127.8, 127.6, 126.9, 118.8, 114.7, 69.2, 62.5, 56.3, 34.8, 30.9, 24.9, 21.9, 13.9. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{28}H_{33}N_2O_4$, 461.2435; found, 461.2421.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(4-hydroxyphenethylamino)-2-oxoethyl)hexanamide (**10**).* ^{1}H NMR (300 MHz, acetone-d_6): δ 8.13 (br s, 1H), 7.51−7.26 (m, 9H), 6.99−6.88 (m, 4H), 6.70 (d, J = 8.5 Hz, 2H), 5.41 (d, J = 7.7 Hz, 1H), 5.12 (s, 2H), 3.50−3.21 (m, 2H), 2.63 (t, J = 7.1 Hz, 2H), 2.29−2.19 (m, 2H), 1.65−1.51 (m, 2H), 1.35−1.22 (m, 4H), 0.92−0.82 (m, 3H). ^{13}C NMR (APT, 126 MHz, DMSO-d_6): δ 171.7, 170.0, 157.6, 155.6, 137.1, 131.4, 129.5, 129.3, 128.4, 128.2, 127.8, 127.6, 115.0, 114.4, 69.2, 55.4, 40.6, 34.9, 34.0, 30.9, 24.9, 21.8, 13.9. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{29}H_{35}N_2O_4$, 475.2591; found, 475.2581.

*(S)-1-(2-(4-(Benzyloxy)phenyl)-2-hexanamidoacetyl)piperidine-4-carboxamide (**11**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.32−8.19 (m, 1H), 7.56−7.16 (m, 9H), 6.97 (d, J = 7.6 Hz, 2H), 5.78 (d, J = 8.0 Hz, 1H), 5.09 (s, 2H), 4.33 (t, J = 14.5 Hz, 1H), 3.89 (t, J = 14.5 Hz, 1H), 3.11−2.54 (m, 2H), 2.35−2.19 (m, 1H), 2.18−2.06 (m, 2H), 1.73−1.08 (m, 10H), 0.82 (t, J = 6.9 Hz, 3H). HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{27}H_{36}N_3O_4$, 466.2700; found, 466.2699.

*(1S,4r)-4-(((S)-2-(4-(Benzyloxy)phenyl)-2-hexanamidoacetamido)methyl)cyclohexanecarboxamide (**13**).* ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44−7.27 (m, 7H), 6.94 (d, J = 8.7 Hz, 2H), 5.68 (s, 1H), 5.05 (s, 2H), 3.11 (s, 2H), 2.21 (t, J = 7.6 Hz, 2H), 2.06−1.84 (m, 3H), 1.75−1.54 (m, 6H), 1.48−1.34 (m, 3H), 1.32−1.19 (m, 4H), 0.86 (t, J = 6.5 Hz, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 177.2, 171.8, 170.1, 159.8, 137.1, 131.6, 128.4, 128.2, 127.8, 127.6, 114.4, 69.1, 55.4, 43.7, 42.8, 37.0, 34.9, 30.9, 29.5, 28.7, 25.0, 21.9, 13.9. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{29}H_{40}N_3O_4$, 494.3013; found, 494.3010.

*(S)-4-(2-(4-(Benzyloxy)phenyl)-2-hexanamidoacetamido)-benzamide (**14**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.45 (s, 1H), 8.51 (d, J = 7.0 Hz, 1H), 7.92−7.72 (m, 4H), 7.62 (d, J = 8.5 Hz, 2H), 7.49−7.20 (m, 7H), 7.00 (d, J = 8.1 Hz, 2H), 5.55 (d, J = 7.1 Hz, 1H), 5.09 (s, 2H), 2.20 (t, J = 7.1 Hz, 2H), 1.58−1.41 (m, 2H), 1.30−1.18 (m, 4H), 0.84 (t, J = 6.3 Hz, 3H). HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{28}H_{32}N_3O_4$, 474.2387; found, 474.2386.

*(S)-2-(2-(4-(Benzyloxy)phenyl)-2-hexanamidoacetamido)-benzamide (**16**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.27 (s, 1H), 8.64 (d, J = 6.4 Hz, 1H), 8.48 (d, J = 8.5 Hz, 1H), 8.25 (s, 2H), 7.80 (d, J = 7.2 Hz, 1H), 7.71 (s, 1H), 7.51−7.28 (m, 7H), 7.10 (t, J = 7.4 Hz, 1H), 7.00 (d, J = 8.7 Hz, 2H), 5.23 (d, J = 6.4 Hz, 1H), 5.09 (s,

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2H), 2.30−2.20 (m, 2H), 1.50 (p, J = 7.2 Hz, 2H), 1.29−1.18 (m, 4H), 0.83 (t, J = 6.7 Hz, 3H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{28}H_{32}N_3O_4$, 474.2387; found, 474.2379.

*(S)-N-(2-(4-(2-Amino-2-oxoethyl)phenylamino)-1-(4-(benzyloxy)phenyl)-2-oxoethyl)hexanamide (**17**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.12 (s, 1H), 8.36 (d, J = 7.7 Hz, 1H), 7.45 (d, J = 7.8 Hz, 2H), 7.28−7.11 (m, 9H), 7.07 (d, J = 8.5 Hz, 2H), 6.82 (d, J = 8.0 Hz, 2H), 5.45 (d, J = 7.7 Hz, 1H), 5.11 (s, 1H), 3.83 (s, 2H), 2.17 (t, J = 7.1 Hz, 2H), 1.47 (p, J = 7.2 Hz, 2H), 1.30−1.15 (m, 4H), 0.84 (t, J = 6.6 Hz, 3H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{29}H_{34}N_3O_4$, 488.2544; found, 488.2549.

*(S)-4-((2-(4-(Benzyloxy)phenyl)-2-hexanamidoacetamido)-methyl)benzamide (**18**).* ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.71 (t, J = 5.9 Hz, 1H), 8.37 (d, J = 7.9 Hz, 1H), 7.90 (s, 2H), 7.76 (d, J = 8.1 Hz, 2H), 7.44 (d, J = 7.3 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.33 (d, J = 8.6 Hz, 2H), 7.31−7.27 (m, 1H), 7.20 (d, J = 8.1 Hz, 2H), 6.98 (d, J = 8.6 Hz, 2H), 5.42 (d, J = 7.8 Hz, 1H), 5.10 (s, 2H), 4.30 (d, J = 5.7 Hz, 2H), 2.18 (t, J = 7.4 Hz, 2H), 1.48 (p, J = 7.4 Hz, 2H), 1.24−1.16 (m, 4H), 0.83 (t, J = 7.1 Hz, 3H). ^{13}C NMR (APT, 126 MHz, DMSO-d_6): δ 171.9, 170.4, 167.6, 157.7, 142.5, 137.1, 132.7, 131.1, 128.4, 128.4, 127.8, 127.6, 127.4, 126.6, 114.5, 69.2, 55.7, 41.7, 34.9, 30.9, 24.9, 21.8, 13.9. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{29}H_{34}N_3O_4$, 488.2544; found, 488.2537.

*(S)-4-(2-(2-(4-(Benzyloxy)phenyl)-2-hexanamidoacetyl)-hydrazinyl)benzamide (**19**).* ^{1}H NMR (300 MHz, acetone-d_6): δ 9.16 (br s, 1H), 7.71 (d, J = 8.8 Hz, 2H), 7.61−7.54 (m, 1H), 7.49 (d, J = 7.2 Hz, 2H), 7.46−7.22 (m, 6H), 7.02 (d, J = 8.8 Hz, 2H), 6.73 (d, J = 8.7 Hz, 2H), 6.28 (s, 2H), 5.59−5.54 (m, 1H), 5.15 (s, 2H), 2.27 (t, J = 7.4 Hz, 2H), 1.66−1.53 (m, 2H), 1.36−1.21 (m, 4H), 0.86 (t, J = 6.9 Hz, 3H). HRMS (ESI): m/z [M−H]$^-$ calcd for $C_{28}H_{31}N_4O_4$, 487.2351; found, 487.2368.

*(S)-4-(2-(2-(4-(Benzyloxy)phenyl)-2-hexanamidoacetamido)-ethyl)benzamide (**20**).* ^{1}H NMR (300 MHz, CD_3OD/D_2O): δ 7.71 (d, J = 8.6 Hz, 2H), 7.44 (d, J = 6.8 Hz, 2H), 7.40−7.28 (m, 3H), 7.25−7.13 (m, 4H), 6.94 (d, J = 8.8 Hz, 2H), 5.27 (s, 1H), 5.10 (s, 2H), 3.63−3.49 (m, 2H), 2.81 (t, J = 6.8 Hz, 2H), 2.25 (t, J = 7.6 Hz, 2H), 1.66−1.53 (m, 2H), 1.39−1.21 (m, 4H), 0.88 (t, J = 6.7 Hz, 3H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{30}H_{36}N_3O_4$, 502.2700; found, 502.2697.

*(S)-4-((2-(4-(Benzyloxy)phenyl)-2-hexanamidoacetamido)-methyl)benzoic Acid (**25**).* ^{1}H NMR (300 MHz, acetone-d_6): δ 7.99−7.88 (m, 3H), 7.55 (d, J = 7.4 Hz, 1H), 7.48 (d, J = 7.1 Hz, 2H), 7.43−7.28 (m, 7H), 6.98 (d, J = 8.8 Hz, 2H), 5.51 (d, J = 7.5 Hz, 1H), 5.13 (s, 2H), 4.48 (d, J = 6.3 Hz, 2H), 2.26 (t, J = 7.5 Hz, 2H), 1.66−1.52 (m, 2H), 1.34−1.22 (m, 4H), 0.86 (t, J = 6.9 Hz, 3H). HRMS (ESI): m/z [M−H]$^-$ calcd for $C_{29}H_{31}N_2O_5$, 487.2238; found, 487.2266.

*(S)-4-((2-Hexanamido-2-phenylacetamido)methyl)benzamide (**27**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.80 (t, J = 5.7 Hz, 1H), 8.46 (d, J = 7.9 Hz, 1H), 7.90 (s, 2H), 7.76 (d, J = 8.0 Hz, 2H), 7.43 (d, J = 7.0 Hz, 2H), 7.37−7.28 (m, 3H), 7.19 (d, J = 8.0 Hz, 2H), 5.51 (d, J = 7.9 Hz, 1H), 4.31 (d, J = 5.6 Hz, 2H), 2.20 (t, J = 7.3 Hz, 2H), 1.49 (p, J = 7.1 Hz, 2H), 1.32−1.14 (m, 4H), 0.83 (t, J = 6.8 Hz, 3H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{22}H_{28}N_3O_3$, 382.2136; found, 382.2143.

*(S)-4-((2-Hexanamido-2-(4-(trifluoromethyl)phenyl)acetamido)-methyl)benzamide (**28**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.92 (t, J = 5.6 Hz, 1H), 8.63 (d, J = 7.9 Hz, 1H), 7.90 (s, 2H), 7.82−7.70 (m, 4H), 7.64 (d, J = 7.8 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 5.63 (d, J = 7.9 Hz, 1H), 4.44−4.21 (m, 2H), 2.21 (t, J = 7.3 Hz, 2H), 1.54−1.41 (m, 2H), 1.32−1.15 (m, 4H), 0.83 (t, J = 6.8 Hz, 3H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{23}H_{27}F_3N_3O_3$, 450.1999; found, 450.1994.

*(S)-4-((2-Hexanamido-3-phenylpropanamido)methyl)-benzamide (**30**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.51 (t, J = 5.6 Hz, 1H), 8.07 (d, J = 8.3 Hz, 1H), 7.90 (s, 2H), 7.78 (d, J = 8.1 Hz, 2H), 7.40−7.13 (m, 7H), 4.61−4.50 (m, 1H), 4.30 (d, J = 5.6 Hz, 2H), 3.05−2.68 (m, 2H), 2.04 (t, J = 7.5 Hz, 2H), 1.45−1.29 (m, 2H), 1.27−0.99 (m, 4H), 0.80 (t, J = 7.1 Hz, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 172.1, 171.4, 167.6, 142.6, 138.0, 132.7, 129.1, 128.0, 127.4, 126.7, 126.2, 54.0, 41.7, 37.7, 35.1, 30.7, 24.9, 21.9, 13.8. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{23}H_{30}N_3O_3$, 396.2282; found, 396.2287.

*(R)-4-((2-(4-(Benzyloxy)phenyl)-2-hexanamidoacetamido)-methyl)benzamide (**31**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.71 (t, J = 5.8 Hz, 1H), 8.38 (d, J = 7.8 Hz, 1H), 7.91 (s, 2H), 7.77 (d, J = 8.3 Hz, 2H), 7.46−7.28 (m, 7H), 7.20 (d, J = 8.2 Hz, 2H), 6.98 (d, J = 8.8 Hz, 2H), 5.43 (d, J = 7.8 Hz, 1H), 5.10 (s, 2H), 4.30 (d, J = 5.9 Hz, 2H), 2.18 (t, J = 7.4 Hz, 2H), 1.48 (p, J = 7.3 Hz, 2H), 1.30−1.16 (m, 4H), 0.83 (t, J = 6.9 Hz, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 171.9, 170.5, 167.6, 157.7, 142.5, 137.1, 132.7, 131.1, 128.4, 128.3, 127.8, 127.6, 127.4, 126.6, 114.5, 69.2, 55.7, 41.7, 34.9, 30.9, 24.9, 21.9, 13.9. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{29}H_{34}N_3O_4$, 488.2544; found, 488.2538.

*(S)-4-((2-Hexanamido-2-(4-(4-(trifluoromethyl)benzyloxy)-phenyl)acetamido)methyl)benzamide (**32**).* ^{1}H NMR (300 MHz, acetone-d_6): δ 7.96 (t, J = 5.7 Hz, 1H), 7.83 (d, J = 8.0 Hz, 2H), 7.73 (q, J = 8.6 Hz, 4H), 7.59 (d, J = 7.8 Hz, 1H), 7.39 (d, J = 8.6 Hz, 2H), 7.27 (d, J = 7.8 Hz, 2H), 6.99 (d, J = 8.4 Hz, 2H), 6.57 (s, 2H), 5.53 (d, J = 7.5 Hz, 1H), 5.25 (s, 2H), 4.45 (d, J = 6.0 Hz, 2H), 2.26 (t, J = 7.5 Hz, 2H), 1.66−1.48 (m, 2H), 1.35−1.20 (m, 4H), 0.85 (t, J = 5.9 Hz, 3H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{30}H_{33}F_3N_3O_4$, 556.2418; found, 556.2423.

*(R)-4-((2-Hexanamido-2-(4-(4-(trifluoromethyl)benzyloxy)-phenyl)acetamido)methyl)benzamide (**33**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.72 (t, J = 5.7 Hz, 1H), 8.38 (d, J = 7.8 Hz, 1H), 7.90 (s, 2H), 7.77 (d, J = 8.1 Hz, 4H), 7.66 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 8.5 Hz, 2H), 7.20 (d, J = 8.1 Hz, 2H), 6.99 (d, J = 8.5 Hz, 2H), 5.43 (d, J = 7.8 Hz, 1H), 5.23 (s, 2H), 4.30 (d, J = 5.7 Hz, 2H), 2.18 (t, J = 7.3 Hz, 2H), 1.48 (p, J = 7.3 Hz, 2H), 1.30−1.14 (m, 4H), 0.83 (t, J = 6.8 Hz, 3H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{30}H_{33}F_3N_3O_4$, 556.2418; found, 556.2406.

*(S)-4-((2-(4-(4-tert-Butylbenzyloxy)phenyl)-2-hexanamidoacetamido)methyl)benzamide (**34**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.66 (t, J = 5.9 Hz, 1H), 8.30 (d, J = 7.5 Hz, 1H), 7.90 (s, 2H), 7.76 (d, J = 7.7 Hz, 2H), 7.45−7.01 (m, 8H), 6.86 (d, J = 7.9 Hz, 2H), 5.36 (d, J = 7.4 Hz, 1H), 5.13 (s, 2H), 4.30 (d, J = 4.8 Hz, 2H), 2.17 (t, J = 6.9 Hz, 2H), 1.55−1.41 (m, 2H), 1.32−1.11 (m, 13H), 0.83 (t, J = 6.9 Hz, 3H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{33}H_{42}N_3O_4$, 544.3170; found, 544.3161.

*(S)-4-((2-(4-(4-Cyanobenzyloxy)phenyl)-2-hexanamidoacetamido)methyl)benzamide (**35**).* ^{1}H NMR (300 MHz, acetone-d_6): δ 7.92 (t, J = 5.9 Hz, 1H), 7.82 (d, J = 6.7 Hz, 2H), 7.70 (d, J = 7.3 Hz, 2H), 7.55 (d, J = 6.7 Hz, 1H), 7.39 (d, J = 7.9 Hz, 2H), 7.27 (d, J = 7.1 Hz, 2H), 7.14 (d, J = 7.7 Hz, 2H), 6.99 (d, J = 7.8 Hz, 2H), 6.53 (br s, 2H), 5.52 (d, J = 5.8 Hz, 1H), 5.26 (s, 2H), 4.45 (d, J = 4.2 Hz, 2H), 2.26 (t, J = 7.6 Hz, 2H), 1.69−1.49 (m, 2H), 1.37−1.20 (m, 4H), 0.86 (t, J = 5.8 Hz, 3H). ^{13}C NMR (APT, 126 MHz, DMSO-d_6): δ 171.9, 170.4, 167.6, 157.3, 143.0, 142.5, 132.7, 132.4, 131.4, 128.0, 127.4, 126.6, 118.7, 114.5, 110.4, 68.2, 55.7, 41.7, 34.8, 30.9, 24.9, 21.8, 13.9. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{30}H_{33}N_4O_4$, 513.2496; found, 513.2499.

*(S)-4-((2-Hexanamido-2-(4-(3-methoxybenzyloxy)phenyl)-acetamido)methyl)benzamide (**36**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.71 (t, J = 5.6 Hz, 1H), 8.37 (d, J = 7.8 Hz, 1H), 7.90 (s, 2H), 7.77 (d, J = 8.0 Hz, 2H), 7.39−7.24 (m, 4H), 7.20 (d, J = 8.0 Hz, 2H), 7.06−6.94 (m, 3H), 6.88 (d, J = 8.4 Hz, 1H), 5.42 (d, J = 7.8 Hz, 1H), 5.08 (s, 2H), 4.30 (d, J = 5.4 Hz, 2H), 3.76 (s, 3H), 2.18 (t, J = 7.2 Hz, 2H), 1.48 (p, J = 7.0 Hz, 2H), 1.30−1.15 (m, 4H), 0.83 (t, J = 6.8 Hz, 3H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{30}H_{36}N_3O_5$, 518.2649; found, 518.2648.

*(S)-4-((2-(4-(2,6-Dichlorobenzyloxy)phenyl)-2-hexanamidoacetamido)methyl)benzamide (**37**).* ^{1}H NMR (300 MHz, acetone-d_6): δ 7.95−7.87 (m, 1H), 7.83 (d, J = 7.3 Hz, 2H), 7.58−7.38 (m, 6H), 7.30 (d, J = 7.4 Hz, 2H), 7.04 (d, J = 7.7 Hz, 2H), 6.52 (s, 2H), 5.53 (d, J = 6.8 Hz, 1H), 5.32 (s, 2H), 4.47 (d, J = 4.7 Hz, 2H), 2.47−2.31 (m, 2H), 1.68−1.49 (m, 2H), 1.37−1.22 (m, 4H), 0.86 (t, J = 5.8 Hz, 3H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{29}H_{32}Cl_2N_3O_4$, 556.1764; found, 556.1754.

*(R)-4-((2-(4-(3,4-Dichlorobenzyloxy)phenyl)-2-hexanamidoacetamido)methyl)benzamide (**38**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.73 (t, J = 5.6 Hz, 1H), 8.39 (d, J = 7.8 Hz, 1H),

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7.91 (s, 2H), 7.77 (d, J = 8.0 Hz, 2H), 7.72 (s, 1H), 7.66 (d, J = 8.2 Hz, 1H), 7.46−7.30 (m, 3H), 7.20 (d, J = 7.9 Hz, 2H), 6.99 (d, J = 8.3 Hz, 2H), 5.43 (d, J = 7.8 Hz, 1H), 5.12 (s, 2H), 4.30 (d, J = 5.3 Hz, 2H), 2.18 (t, J = 7.2 Hz, 2H), 1.48 (p, J = 7.2 Hz, 2H), 1.31−1.13 (m, 4H), 0.83 (t, J = 6.7 Hz, 3H). HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{29}H_{32}Cl_2N_3O_4$, 556.1764; found, 556.1755.

*4-(((S)-2-((S)-2-Acetamidohexanamido)-2-(4-(benzyloxy)phenyl)acetamido)methyl)benzamide (**39**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.70 (t, J = 5.9 Hz, 1H), 8.36 (d, J = 7.5 Hz, 1H), 7.99 (d, J = 8.0 Hz, 1H), 7.90 (s, 2H), 7.76 (d, J = 7.9 Hz, 2H), 7.49−7.25 (m, 7H), 7.18 (d, J = 7.9 Hz, 2H), 6.99 (d, J = 8.3 Hz, 2H), 5.39 (d, J = 7.5 Hz, 1H), 5.11 (s, 2H), 4.40−4.23 (m, 3H), 1.83 (s, 3H), 1.69−1.21 (m, 6H), 0.84 (t, J = 6.5 Hz, 3H). HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{31}H_{37}N_4O_5$, 545.2758; found, 545.2740.

*(S)-4-((2-(4-(Benzyloxy)phenyl)-2-(cyclobutanecarboxamido)acetamido)methyl)benzamide (**41**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.71 (t, J = 5.8 Hz, 1H), 8.21 (d, J = 7.8 Hz, 1H), 7.91 (s, 2H), 7.77 (d, J = 8.2 Hz, 2H), 7.50−7.26 (m, 7H), 7.19 (d, J = 8.2 Hz, 2H), 6.98 (d, J = 8.8 Hz, 2H), 5.42 (d, J = 7.8 Hz, 1H), 5.10 (s, 2H), 4.30 (d, J = 5.8 Hz, 2H), 3.21 (p, J = 8.0 Hz, 1H), 2.29−1.66 (m, 6H). HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{28}H_{30}N_3O_4$, 472.2231; found, 472.2228.

*(S)-4-((2-(4-(Benzyloxy)phenyl)-2-pentanamidoacetamido)methyl)benzamide (**42**).* ^{1}H NMR (300 MHz, acetone-d_6): δ 7.90 (t, J = 6.7 Hz, 1H), 7.82 (d, J = 7.5 Hz, 2H), 7.53 (d, J = 7.6 Hz, 1H), 7.47 (d, J = 7.4 Hz, 2H), 7.44−7.30 (m, 5H), 7.27 (d, J = 7.9 Hz, 2H), 6.97 (d, J = 7.1 Hz, 2H), 6.56 (s, 2H), 5.51 (d, J = 6.3 Hz, 1H), 5.12 (s, 2H), 4.45 (d, J = 5.7 Hz, 2H), 2.26 (t, J = 6.7 Hz, 2H), 1.56 (p, J = 7.4 Hz, 2H), 1.38−1.23 (m, 2H), 0.87 (t, J = 7.2 Hz, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 171.9, 170.5, 167.6, 157.7, 142.5, 137.1, 132.7, 131.0, 128.44, 128.42, 127.8, 127.6, 127.4, 126.6, 114.5, 69.2, 55.7, 41.7, 34.6, 27.4, 21.8, 13.8. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{28}H_{32}N_3O_4$, 474.2387; found, 474.2393.

*(S)-4-((2-(4-(Benzyloxy)phenyl)-2-heptanamidoacetamido)methyl)benzamide (**43**).* ^{1}H NMR (300 MHz, acetone-d_6): δ 7.95−7.88 (m, 1H), 7.82 (d, J = 7.0 Hz, 2H), 7.56−7.51 (m, 1H), 7.49 (d, J = 7.0 Hz, 2H), 7.43−7.30 (m, 5H), 7.28 (d, J = 7.7 Hz, 2H), 6.97 (d, J = 9.0 Hz, 2H), 6.52 (s, 2H), 5.53−5.47 (m, 1H), 5.12 (s, 2H), 4.45 (d, J = 6.2 Hz, 2H), 2.30−2.22 (m, 2H), 1.65−1.50 (m, 2H), 1.34−1.22 (m, 6H), 0.92−0.82 (m, 3H). ^{13}C NMR (APT, 126 MHz, DMSO-d_6): δ 171.9, 170.4, 167.6, 157.7, 142.5, 137.1, 132.7, 131.1, 128.43, 128.41, 127.8, 127.6, 127.4, 126.6, 114.5, 69.2, 55.7, 41.7, 34.9, 31.0, 28.3, 25.2, 22.0, 13.9. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{30}H_{36}N_3O_4$, 502.2700; found, 502.2704.

*(S)-4-((2-(4-(Benzyloxy)phenyl)-2-octanamidoacetamido)methyl)benzamide (**44**).* ^{1}H NMR (300 MHz, acetone-d_6): δ 7.90 (t, J = 5.7 Hz, 1H), 7.82 (d, J = 8.1 Hz, 2H), 7.53 (d, J = 7.2 Hz, 1H), 7.47 (d, J = 7.4 Hz, 2H), 7.43−7.33 (m, 5H), 7.28 (d, J = 8.1 Hz, 2H), 6.97 (d, J = 8.6 Hz, 2H), 6.52 (s, 2H), 5.50 (d, J = 7.4 Hz, 1H), 5.12 (s, 2H), 4.45 (d, J = 6.0 Hz, 2H), 2.26 (t, J = 7.4 Hz, 2H), 1.66−1.51 (m, 2H), 1.36−1.22 (m, 8H), 0.86 (t, J = 5.7 Hz, 3H). HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{31}H_{38}N_3O_4$, 516.2857; found, 516.2833.

*(S)-4-(4-(4-(Benzyloxy)phenyl)-3,6-dioxo-8,11-dioxa-2,5-diazadodecyl)benzamide (**47**).* ^{1}H NMR (300 MHz, CD_3OD/D_2O): δ 7.73 (d, J = 8.1 Hz, 2H), 7.45−7.29 (m, 7H), 7.24 (d, J = 8.1 Hz, 2H), 7.01 (d, J = 8.5 Hz, 2H), 5.40 (s, 1H), 5.12 (s, 2H), 4.55−4.30 (m, 2H), 4.15−3.96 (m, 2H), 3.78−3.48 (m, 4H), 3.21 (s, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 169.9, 168.7, 167.6, 157.9, 142.3, 137.0, 132.8, 130.8, 128.4, 128.3, 127.8, 127.6, 127.5, 126.7, 114.7, 71.0, 70.2, 69.6, 69.2, 58.1, 55.1, 41.8. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{28}H_{32}N_3O_6$, 506.2286; found, 506.2280.

*4-(((2S)-2-(4-(Benzyloxy)phenyl)-2-(2-hydroxypentanamido)acetamido)methyl)benzamide (**48**).* ^{1}H NMR (300 MHz, CD_3OD/D_2O): δ 7.75 (d, J = 7.9 Hz, 2H), 7.46−7.30 (m, 7H), 7.24 (d, J = 7.7 Hz, 2H), 7.00 (d, J = 8.1 Hz, 2H), 5.41 (s, 1H), 5.11 (s, 2H), 4.38 (s, 2H), 4.10−4.02 (m, 1H), 1.76−1.26 (m, 4H), 0.91 (t, J = 7.6 Hz, 3H). HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{28}H_{32}N_3O_5$, 490.2336; found, 490.2343.

*(S)-4-((2-(6-Aminohexanamido)-2-(4-(benzyloxy)phenyl)acetamido)methyl)benzamide (**49**).* ^{1}H NMR (300 MHz, CD_3OD): δ 8.72 (t, J = 5.8 Hz, 1H), 8.36 (d, J = 6.5 Hz, 1H), 7.76 (d, J = 8.2 Hz, 2H), 7.47−7.29 (m, 7H), 7.26 (d, J = 8.2 Hz, 2H), 7.00 (d, J = 8.8 Hz, 2H), 5.40−5.34 (m, 1H), 5.10 (s, 2H), 4.54−4.30 (m, 2H), 2.90 (t, J = 7.6 Hz, 2H), 2.41−2.22 (m, 2H), 1.74−1.56 (m, 4H), 1.46−1.32 (m, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 171.7, 170.4, 167.6, 157.8, 142.5, 137.1, 132.7, 131.0, 128.5, 128.4, 127.8, 127.6, 127.4, 126.6, 114.6, 69.2, 55.8, 41.7, 34.6, 26.8, 25.5, 24.7. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{29}H_{35}N_4O_4$, 503.2653; found, 503.2663.

*(S)-4-(Aminomethyl)-N-(1-(4-(benzyloxy)phenyl)-2-(4-carbamoylbenzylamino)-2-oxoethyl)benzamide (**50**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.83 (d, J = 7.7 Hz, 1H), 8.77 (t, J = 5.8 Hz, 1H), 8.29 (br s, 3H), 7.97 (d, J = 8.2 Hz, 2H), 7.92 (s, 2H), 7.78 (d, J = 8.2 Hz, 2H), 7.53 (d, J = 8.3 Hz, 2H), 7.48−7.29 (m, 7H), 7.23 (d, J = 8.2 Hz, 2H), 7.01 (d, J = 8.7 Hz, 2H), 5.65 (d, J = 7.5 Hz, 1H), 5.12 (s, 2H), 4.35 (d, J = 5.6 Hz, 2H), 4.11 (s, 2H). HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{31}H_{31}N_4O_4$, 523.2340; found, 523.2366.

*(S)-4-((2-(4-(Benzyloxy)phenyl)-2-(3-phenylpropanamido)acetamido)methyl)benzamide (**51**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.73 (t, J = 6.0 Hz, 1H), 8.46 (d, J = 7.8 Hz, 1H), 7.91 (s, 2H), 7.78 (d, J = 8.3 Hz, 2H), 7.46−7.17 (m, 14H), 6.97 (d, J = 8.8 Hz, 2H), 5.43 (d, J = 7.8 Hz, 1H), 5.10 (s, 2H), 4.31 (d, J = 5.7 Hz, 2H), 2.80 (t, J = 7.8 Hz, 2H), 2.59−2.51 (m, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 171.1, 170.3, 167.6, 157.7, 142.5, 141.3, 137.1, 132.7, 131.0, 128.4, 128.4, 128.2, 127.8, 127.6, 127.4, 126.7, 125.8, 114.5, 69.2, 55.8, 41.7, 36.5, 31.1. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{32}H_{32}N_3O_4$, 522.2387; found, 522.2395.

*(S,E)-4-((2-(4-(Benzyloxy)phenyl)-2-cinnamamidoacetamido)methyl)benzamide (**52**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.83 (t, J = 5.6 Hz, 1H), 8.70 (d, J = 7.7 Hz, 1H), 7.91 (s, 2H), 7.78 (d, J = 7.9 Hz, 2H), 7.55 (d, J = 6.7 Hz, 2H), 7.45−7.29 (m, 11H), 7.22 (d, J = 7.9 Hz, 2H), 7.01 (d, J = 8.3 Hz, 2H), 6.94 (d, J = 15.8 Hz, 1H), 5.57 (d, J = 7.7 Hz, 1H), 5.11 (s, 2H), 4.33 (d, J = 4.3 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 170.3, 167.6, 164.5, 157.9, 142.5, 139.1, 137.1, 134.9, 132.8, 130.9, 129.5, 128.9, 128.5, 128.4, 127.8, 127.6, 127.5, 126.7, 122.1, 114.6, 69.2, 56.0, 41.8. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{32}H_{30}N_3O_4$, 520.2231; found, 520.2228.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(4-carbamoylbenzylamino)-2-oxoethyl)thiophene-2-carboxamide (**54**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.84 (d, J = 7.9 Hz, 1H), 8.76 (t, J = 5.8 Hz, 1H), 8.02 (d, J = 3.4 Hz, 1H), 7.91 (s, 2H), 7.82−7.73 (m, 3H), 7.46−7.29 (m, 7H), 7.23 (d, J = 8.1 Hz, 2H), 7.16−7.12 (m, 1H), 7.01 (d, J = 8.5 Hz, 2H), 5.61 (d, J = 7.6 Hz, 1H), 5.12 (s, 2H), 4.34 (d, J = 5.5 Hz, 2H). HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{28}H_{26}N_3O_4S$, 500.1639; found, 500.1641.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(4-carbamoylbenzylamino)-2-oxoethyl)benzo[b]thiophene-2-carboxamide (**55**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.12 (d, J = 7.5 Hz, 1H), 8.80 (t, J = 5.8 Hz, 1H), 8.38 (s, 1H), 8.01 (d, J = 8.5 Hz, 1H), 7.91 (s, 2H), 7.79 (d, J = 8.2 Hz, 2H), 7.49−7.29 (m, 10H), 7.24 (d, J = 8.2 Hz, 2H), 7.03 (d, J = 8.7 Hz, 2H), 5.64 (d, J = 7.6 Hz, 1H), 5.13 (s, 2H), 4.36 (d, J = 5.7 Hz, 2H). HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{32}H_{28}N_3O_4S$, 550.1795; found, 550.1792.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(4-carbamoylbenzylamino)-2-oxoethyl)-2,2′-bithiophene-5-carboxamide (**56**).* ^{1}H NMR (300 MHz, acetone-d_6): δ 8.11−7.99 (m, 2H), 7.86−7.79 (m, 3H), 7.52−7.28 (m, 11H), 7.26 (d, J = 3.9 Hz, 1H), 7.11 (dd, J = 5.1, 3.7 Hz, 1H), 7.01 (d, J = 8.8 Hz, 2H), 6.55 (br s, 2H), 5.69 (d, J = 7.3 Hz, 1H), 5.14 (s, 2H), 4.50 (d, J = 6.0 Hz, 2H). HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{32}H_{28}N_3O_4S_2$, 582.1516; found, 582.1513.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(4-carbamoylbenzylamino)-2-oxoethyl)-4-phenoxybenzamide (**57**).* ^{1}H NMR (300 MHz, acetone-d_6): δ 8.08−7.93 (m, 4H), 7.83 (d, J = 8.1 Hz, 2H), 7.52−7.26 (m, 11H), 7.20 (t, J = 7.3 Hz, 1H), 7.08 (d, J = 7.8 Hz, 2H), 7.05−6.96 (m, 4H), 6.53 (br s, 2H), 5.72 (d, J = 7.0 Hz, 1H), 5.13 (s, 2H), 4.49 (d, J = 5.9 Hz, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 170.3, 168.3, 167.6, 159.5, 157.9, 155.7, 142.6, 137.1, 132.7, 130.6, 130.2, 130.0, 129.0, 128.6, 128.4, 127.8, 127.7, 127.4, 126.7, 124.2, 119.4, 117.3, 114.5, 69.2, 56.7, 41.9. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{36}H_{32}N_3O_5$, 586.2336; found, 586.2329.

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(S)-4-(Benzyloxy)-N-(1-(4-(benzyloxy)phenyl)-2-(4-carbamoylbenzylamino)-2-oxoethyl)benzamide (**58**). ^{1}H NMR (300 MHz, acetone-d_6): δ 7.99 (t, J = 5.9 Hz, 1H), 7.96−7.88 (m, 3H), 7.83 (d, J = 8.1 Hz, 2H), 7.51−7.34 (m, 12H), 7.30 (d, J = 8.3 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.6 Hz, 2H), 6.53 (s, 2H), 5.70 (d, J = 7.1 Hz, 1H), 5.19 (s, 2H), 5.13 (s, 2H), 4.49 (d, J = 6.0 Hz, 2H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{37}H_{34}N_3O_5$, 600.2493; found, 600.2474.

(S)-4-((2-(4-(Benzyloxy)phenyl)-2-(2-(biphenyl-4-yl)acetamido)-acetamido)methyl)benzamide (**59**). ^{1}H NMR (300 MHz, acetone-d_6): δ 7.94 (t, J = 6.0 Hz, 1H), 7.82 (d, J = 8.1 Hz, 2H), 7.72−7.67 (m, 1H), 7.64 (d, J = 7.4 Hz, 2H), 7.58 (d, J = 8.0 Hz, 2H), 7.50−7.32 (m, 12H), 7.26 (d, J = 8.2 Hz, 2H), 6.96 (d, J = 8.6 Hz, 2H), 6.47 (s, 2H), 5.48 (d, J = 7.4 Hz, 1H), 5.11 (s, 2H), 4.44 (d, J = 5.8 Hz, 2H), 3.67 (s, 2H). HRMS (ESI): m/z [M + Na]$^+$ calcd for $C_{37}H_{33}N_3NaO_4$, 606.2363; found, 606.2376.

(S)-3-(2-(4-(2,6-Dichlorobenzyloxy)phenyl)-2-hexanamidoacetamido)benzamide (**60**). ^{1}H NMR (300 MHz, CD_3OD): δ 8.04 (s, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.57 (d, J = 8.0 Hz, 1H), 7.50−7.41 (m, 4H), 7.41−7.31 (m, 2H), 7.06 (d, J = 8.7 Hz, 2H), 5.54 (s, 1H), 5.30 (s, 2H), 2.31 (t, J = 7.5 Hz, 2H), 1.69 (p, J = 7.4 Hz, 2H), 1.40−1.25 (m, 4H), 0.90 (t, J = 6.9 Hz, 3H). ^{13}C NMR (APT, 75 MHz, CD_3OD): δ 176.0, 172.1, 171.4, 160.5, 139.9, 138.0, 136.0, 133.5, 132.0, 131.1, 130.2, 130.0, 129.7, 124.6, 124.3, 120.6, 116.2, 66.3, 58.9, 49.0, 36.6, 32.5, 26.6, 23.5, 14.3. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{28}H_{30}Cl_2N_3O_4$, 542.1608; found, 542.1590.

(S)-3-(2-(4-(Benzyloxy)phenyl)-2-octanamidoacetamido)-benzamide (**61**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.35 (s, 1H), 8.48 (d, J = 7.7 Hz, 1H), 8.01 (s, 1H), 7.91 (s, 2H), 7.74 (d, J = 8.1 Hz, 1H), 7.52 (d, J = 7.9 Hz, 1H), 7.45−7.26 (m, 8H), 7.00 (d, J = 8.8 Hz, 2H), 5.54 (d, J = 7.8 Hz, 1H), 5.09 (s, 2H), 2.20 (t, J = 7.4 Hz, 2H), 1.48 (p, J = 7.1 Hz, 2H), 1.33−1.16 (m, 8H), 0.84 (t, J = 6.7 Hz, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 172.2, 169.4, 167.7, 157.9, 138.8, 137.0, 135.1, 130.5, 130.2, 128.6, 128.4, 127.8, 127.6, 125.1, 121.8, 118.7, 114.7, 69.2, 56.4, 34.8, 31.2, 28.6, 28.4, 25.2, 22.0, 13.9. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{30}H_{36}N_3O_4$, 502.2706; found, 502.2684.

(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-2-naphthamide (**62**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.45 (s, 1H), 9.06 (d, J = 7.2 Hz, 1H), 8.59 (s, 1H), 8.09−7.89 (m, 7H), 7.79 (d, J = 8.0 Hz, 1H), 7.67−7.57 (m, 2H), 7.55 (d, J = 8.6 Hz, 2H), 7.49−7.27 (m, 7H), 7.05 (d, J = 8.5 Hz, 2H), 5.81 (d, J = 7.2 Hz, 1H), 5.12 (s, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 169.3, 167.8, 166.5, 158.1, 138.9, 137.0, 135.1, 134.2, 132.0, 131.0, 129.6, 129.5, 129.3, 128.9, 128.6, 128.4, 128.1, 127.8, 127.7, 127.6, 126.7, 124.6, 124.5, 122.1, 121.8, 118.8, 114.7, 69.2, 57.5. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{33}H_{28}N_3O_4$, 530.2074; found, 530.2071.

(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-carboxamide (**63**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.36 (s, 1H), 8.71 (d, J = 7.1 Hz, 1H), 8.03 (s, 1H), 7.92 (s, 2H), 7.76 (d, J = 7.8 Hz, 1H), 7.57−7.27 (m, 12H), 7.02 (d, J = 8.2 Hz, 2H), 5.70 (d, J = 7.1 Hz, 1H), 5.10 (s, 2H), 4.27 (s, 4H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{31}H_{28}N_3O_6$, 538.1973; found, 538.1969.

(S)-N-(2-(3-Carbamoylphenylamino)-1-(4-(2,6-dichlorobenzyloxy)phenyl)-2-oxoethyl)-4-phenoxybenzamide (**64**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.42 (s, 1H), 8.86 (d, J = 7.4 Hz, 1H), 8.06 (s, 1H), 7.99 (d, J = 8.7 Hz, 2H), 7.93 (s, 2H), 7.79 (d, J = 8.3 Hz, 1H), 7.59−7.30 (m, 9H), 7.21 (t, J = 7.5 Hz, 1H), 7.12−7.06 (m, 4H), 7.03 (d, J = 8.8 Hz, 2H), 5.76 (d, J = 7.2 Hz, 1H), 5.22 (s, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 169.2, 167.8, 165.7, 159.6, 158.2, 155.6, 138.9, 136.0, 135.1, 131.7, 131.6, 130.2, 130.0, 129.9, 129.5, 129.3, 129.2, 128.8, 128.6, 128.5, 124.8, 119.5, 118.8, 117.3, 114.4, 64.9, 57.5. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{35}H_{28}Cl_2N_3O_5$, 640.1401; found, 640.1389.

(S)-N-(2-(3-Carbamoylphenylamino)-1-(4-(3-methoxybenzyloxy)phenyl)-2-oxoethyl)-4-phenoxybenzamide (**65**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.40 (s, 1H), 8.83 (d, J = 7.3 Hz, 1H), 8.04 (s, 1H), 7.97 (d, J = 8.6 Hz, 2H), 7.93 (s, 2H), 7.77 (d, J = 8.0 Hz, 1H), 7.56−7.45 (m, 3H), 7.42 (d, J = 7.7 Hz, 2H), 7.37 (d, J = 7.7 Hz, 1H), 7.35−7.25 (m, 2H), 7.21 (t, J = 7.4 Hz, 1H), 7.08 (d, J = 7.8 Hz, 2H), 7.05−6.96 (m, 5H), 6.88 (d, J = 8.1 Hz, 1H), 5.73 (d, J = 7.2 Hz, 1H), 5.08 (s, 2H), 3.74 (s, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 169.2, 167.8, 165.6, 159.6, 159.3, 158.0, 157.9, 138.9, 138.6, 135.1, 133.9, 130.2, 130.0, 129.9, 129.5, 129.4, 129.2, 128.6, 124.3, 121.8, 119.6, 119.4, 118.8, 117.2, 114.7, 113.2, 113.1, 69.0, 57.4, 55.0. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{36}H_{32}N_3O_6$, 602.2286; found, 602.2268.

(S)-N-(2-(3-Carbamoylphenylamino)-2-oxo-1-(4-phenoxyphenyl)ethyl)-4-phenoxybenzamide (**66**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.48 (s, 1H), 8.92 (d, J = 7.6 Hz, 1H), 8.06 (s, 1H), 7.99 (d, J = 8.2 Hz, 2H), 7.95 (s, 2H), 7.79 (d, J = 7.7 Hz, 1H), 7.59 (d, J = 8.4 Hz, 2H), 7.55−7.28 (m, 7H), 7.25−6.95 (m, 9H), 5.81 (d, J = 7.4 Hz, 1H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 169.0, 167.7, 165.7, 159.6, 156.4, 155.6, 138.8, 135.1, 132.5, 132.0, 130.2, 130.0, 129.9, 129.9, 129.7, 128.6, 128.4, 124.3, 123.6, 122.2, 121.8, 119.4, 118.7, 118.4, 117.2, 57.4. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{34}H_{28}N_3O_5$, 558.2023; found, 558.2021.

(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-4-phenoxybenzamide (**67**). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.40 (s, 1H), 8.84 (d, J = 7.4 Hz, 1H), 8.04 (s, 1H), 7.97 (d, J = 8.8 Hz, 2H), 7.95 (d, J = 8.9 Hz, 1H), 7.93 (s, 2H), 7.77 (d, J = 8.0 Hz, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 8.7 Hz, 2H), 7.46−7.29 (m, 7H), 7.21 (t, J = 6.9 Hz, 1H), 7.08 (d, J = 8.5 Hz, 2H), 7.05−6.99 (m, 4H), 5.74 (d, J = 7.3 Hz, 1H), 5.10 (s, 2H). ^{13}C NMR (APT, 126 MHz, DMSO-d_6): δ 172.1, 169.2, 167.8, 159.6, 158.0, 155.6, 138.9, 137.0, 135.1, 130.2, 130.0, 129.7, 129.4, 129.2, 128.6, 128.5, 128.4, 127.8, 127.6, 124.2, 122.1, 121.8, 119.4, 117.2, 114.7, 69.2, 57.4. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{35}H_{30}N_3O_5$, 572.2180; found, 572.2162.

(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-3-phenoxybenzamide (**68**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.39 (s, 1H), 8.99 (d, J = 7.1 Hz, 1H), 8.03 (s, 1H), 7.93 (s, 2H), 7.75 (t, J = 7.0 Hz, 2H), 7.59−7.30 (m, 13H), 7.23−7.12 (m, 2H), 7.08−6.97 (m, 4H), 5.71 (d, J = 7.1 Hz, 1H), 5.10 (s, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 169.1, 167.8, 165.6, 158.1, 156.5, 138.9, 137.0, 135.7, 135.1, 130.1, 129.9, 129.4, 129.3, 128.6, 128.4, 127.8, 127.6, 123.6, 122.9, 122.1, 121.8, 118.8, 118.6, 117.9, 114.7, 69.2, 57.5. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{35}H_{30}N_3O_5$, 572.2180; found, 572.2162.

(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-4-(2-methoxyphenoxy)benzamide (**69**). ^{1}H NMR (300 MHz, acetone-d_6): δ 9.66 (s, 1H), 8.16 (s, 2H), 8.03 (d, J = 6.7 Hz, 1H), 7.94 (d, J = 8.0 Hz, 2H), 7.85 (d, J = 7.9 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.56−7.28 (m, 9H), 7.24 (d, J = 7.6 Hz, 1H), 7.17 (d, J = 8.0 Hz, 1H), 7.11 (d, J = 7.6 Hz, 1H), 7.06−6.96 (m, 3H), 6.87 (d, J = 8.3 Hz, 2H), 5.82 (d, J = 7.0 Hz, 1H), 5.13 (s, 2H), 3.76 (s, 3H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{36}H_{32}N_3O_6$, 602.2286; found, 602.2302.

(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-4-(4-cyanophenoxy)benzamide (**70**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.41 (s, 1H), 8.94 (d, J = 7.2 Hz, 1H), 8.09−7.99 (m, 2H), 7.92 (s, 2H), 7.88 (d, J = 8.5 Hz, 2H), 7.77 (d, J = 7.8 Hz, 1H), 7.57−7.27 (m, 10H), 7.23−7.14 (m, 4H), 7.03 (d, J = 8.4 Hz, 2H), 5.74 (d, J = 7.0 Hz, 1H), 5.11 (s, 2H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{36}H_{29}N_4O_5$, 597.2132; found, 597.2121.

(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-4-(4-(trifluoromethyl)phenoxy)benzamide (**71**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.41 (s, 1H), 8.93 (d, J = 7.3 Hz, 1H), 8.03 (d, J = 8.5 Hz, 2H), 8.01−7.97 (m, 1H), 7.93 (s, 2H), 7.82−7.73 (m, 3H), 7.56−7.29 (m, 10H), 7.26−7.14 (m, 4H), 7.03 (d, J = 8.7 Hz, 2H), 5.75 (d, J = 7.1 Hz, 1H), 5.11 (s, 2H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{36}H_{29}F_3N_3O_5$, 640.2054; found, 640.2041.

(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-4-(4-chlorophenoxy)benzamide (**72**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.40 (s, 1H), 8.87 (d, J = 7.2 Hz, 1H), 8.04 (s, 1H), 7.99 (d, J = 8.5 Hz, 2H), 7.93 (s, 2H), 7.77 (d, J = 8.0 Hz, 1H), 7.57−7.27 (m, 11H), 7.11 (d, J = 8.7 Hz, 2H), 7.08−6.98 (m, 4H), 5.74 (d, J = 7.2 Hz, 1H), 5.10 (s, 2H). ^{13}C NMR (APT, 75

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MHz, DMSO-d_6): δ 169.2, 167.8, 165.6, 159.1, 158.0, 154.7, 138.9, 137.0, 135.1, 130.1, 129.6, 129.4, 129.2, 128.9, 128.6, 128.4, 128.0, 127.8, 127.6, 122.1, 121.8, 121.1, 118.8, 117.5, 114.7, 69.2, 57.4. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{35}H_{29}ClN_3O_5$, 606.1790; found, 606.1790.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-4-(3-chlorophenoxy)benzamide (**73**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.41 (s, 1H), 8.89 (d, J = 6.7 Hz, 1H), 8.13−7.89 (m, 3H), 7.78 (d, J = 7.3 Hz, 1H), 7.60−7.21 (m, 11H), 7.17−6.93 (m, 6H), 5.74 (d, J = 6.6 Hz, 1H), 5.11 (s, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 169.2, 167.8, 165.6, 158.6, 158.1, 157.0, 138.9, 137.0, 135.1, 134.1, 131.6, 130.2, 129.6, 129.3, 129.2, 128.6, 128.4, 127.8, 127.6, 124.0, 122.1, 121.8, 119.1, 118.8, 118.0, 117.8, 114.7, 69.2, 57.5. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{35}H_{29}ClN_3O_5$, 606.1790; found, 606.1761.

*(S)-4-(Benzo[d][1,3]dioxol-5-yloxy)-N-(1-(4-(benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)benzamide (**74**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.39 (s, 1H), 8.80 (d, J = 7.2 Hz, 1H), 8.03 (s, 1H), 8.00−7.84 (m, 3H), 7.77 (d, J = 7.7 Hz, 1H), 7.57−7.27 (m, 10H), 7.08−6.90 (m, 5H), 6.79 (s, 1H), 6.56 (d, J = 8.3 Hz, 1H), 6.06 (s, 2H), 5.73 (d, J = 7.2 Hz, 1H), 5.10 (s, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 168.7, 167.8, 165.5, 160.6, 158.2, 150.6, 149.6, 146.9, 140.1, 137.1, 133.2, 129.9, 129.2, 128.4, 127.9, 127.8, 127.6, 122.4, 118.6, 116.2, 114.7, 114.1, 112.6, 108.6, 102.6, 69.2, 56.5. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{36}H_{30}N_3O_7$, 638.1898; found, 638.1880.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-4-(2,3-dihydrobenzo[b][1,4]dioxin-6-yloxy)benzamide (**75**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.39 (s, 1H), 8.79 (d, J = 7.3 Hz, 1H), 8.03 (s, 1H), 7.99−7.88 (m, 3H), 7.76 (d, J = 7.9 Hz, 1H), 7.57−7.27 (m, 10H), 7.06−6.99 (m, 2H), 6.96 (d, J = 8.5 Hz, 2H), 6.90 (d, J = 8.7 Hz, 1H), 6.63 (s, 1H), 6.57 (d, J = 8.5 Hz, 1H), 5.72 (d, J = 7.0 Hz, 1H), 5.10 (s, 2H), 4.25 (s, 4H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{37}H_{32}N_3O_7$, 630.2235; found, 630.2220.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-4-(3-oxo-2,3-dihydro-1H-inden-4-yloxy)benzamide (**76**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.40 (s, 1H), 8.87 (d, J = 7.1 Hz, 1H), 8.03 (s, 1H), 7.95 (d, J = 8.5 Hz, 2H), 7.92 (s, 2H), 7.77 (d, J = 7.6 Hz, 1H), 7.57−7.27 (m, 12H), 7.11 (d, J = 8.0 Hz, 1H), 7.07−6.95 (m, 3H), 5.73 (d, J = 7.2 Hz, 1H), 5.10 (s, 2H), 3.19−3.07 (m, 2H), 2.67−1.57 (m, 2H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{38}H_{32}N_3O_6$, 626.2286; found, 626.2268.

*(S)-4-Benzoyl-N-(1-(4-(benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)benzamide (**77**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.44 (s, 1H), 9.15 (d, J = 7.2 Hz, 1H), 8.08 (d, J = 8.1 Hz, 2H), 8.05 (s, 1H), 7.93 (s, 2H), 7.83−7.72 (m, 5H), 7.69 (d, J = 7.2 Hz, 1H), 7.59 (d, J = 7.5 Hz, 2H), 7.56−7.48 (m, 3H), 7.46−7.29 (m, 6H), 7.04 (d, J = 8.4 Hz, 2H), 5.77 (d, J = 7.1 Hz, 1H), 5.11 (s, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 195.4, 169.0, 167.8, 165.8, 158.1, 139.3, 138.9, 137.1, 137.0, 136.7, 135.1, 133.0, 129.7, 129.4, 129.3, 129.2, 128.7, 128.6, 128.4, 128.0, 127.8, 127.6, 122.2, 121.8, 118.8, 114.7, 69.2, 57.6. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{36}H_{30}N_3O_5$, 584.2180; found, 584.2170.

*(S)-4-Benzoyl-N-(2-(3-carbamoylphenylamino)-1-(4-(3-methoxybenzyloxy)phenyl)-2-oxoethyl)benzamide (**78**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.44 (s, 1H), 9.15 (d, J = 7.1 Hz, 1H), 8.08 (d, J = 8.2 Hz, 2H), 8.05 (s, 1H), 7.93 (s, 2H), 7.83−7.66 (m, 5H), 7.77 (d, J = 7.1 Hz, 1H), 7.59 (d, J = 7.4 Hz, 2H), 7.57−7.48 (m, 2H), 7.41−7.24 (m, 4H), 7.03 (d, J = 8.8 Hz, 2H), 7.01−6.97 (m, 1H), 6.88 (d, J = 8.1 Hz, 1H), 5.77 (d, J = 7.1 Hz, 1H), 5.09 (s, 2H), 3.74 (s, 3H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 195.4, 169.1, 167.8, 165.8, 159.3, 158.1, 139.3, 138.9, 138.9, 138.6, 137.1, 136.7, 135.2, 135.1, 133.0, 129.7, 129.6, 129.3, 129.3, 128.7, 128.0, 122.1, 121.8, 119.6, 118.8, 114.7, 113.1, 113.1, 69.0, 57.6, 55.0. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{37}H_{32}N_3O_6$, 614.2286; found, 614.2278.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-4-(morpholine-4-carbonyl)benzamide (**79**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.41 (s, 1H), 9.02 (d, J = 7.2 Hz, 1H), 8.09−8.02 (m, 3H), 7.99 (d, J = 8.1 Hz, 2H), 7.93 (s, 2H), 7.77 (d, J = 8.0 Hz, 1H), 7.57−7.27 (m, 9H), 7.03 (d, J = 8.6 Hz, 2H), 5.75 (d, J = 7.3 Hz, 1H), 5.11 (s, 2H), 3.80−3.40 (m, 8H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 169.1, 168.4, 167.8, 165.9, 158.1, 138.9, 138.3, 137.0, 135.1, 134.6, 129.5, 129.2, 128.6, 128.4, 128.0, 127.8, 127.6, 126.8, 122.1, 121.8, 118.8, 114.7, 69.2, 66.0, 57.5. Signals of C-3 and C-5 of morpholine were not observed in ^{13}C NMR. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{34}H_{33}N_4O_6$, 593.2395; found, 593.2982.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-3-(morpholine-4-carbonyl)benzamide (**80**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.42 (s, 1H), 9.06 (d, J = 7.1 Hz, 1H), 8.48 (s, 1H), 8.09−7.96 (m, 3H), 7.93 (s, 2H), 7.77 (d, J = 7.7 Hz, 1H), 7.58−7.27 (m, 10H), 7.03 (d, J = 8.6 Hz, 2H), 5.75 (d, J = 7.0 Hz, 1H), 5.11 (s, 2H), 3.80−3.41 (m, 8H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 169.1, 168.5, 167.8, 165.7, 158.1, 138.9, 137.0, 135.6, 135.1, 134.0, 129.7, 129.5, 129.3, 128.9, 128.6, 128.4, 128.4, 127.8, 127.6, 126.4, 122.2, 121.8, 118.8, 114.7, 69.2, 66.1, 57.5. Signals of C-3 and C-5 of morpholine were not observed in ^{13}C NMR. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{34}H_{33}N_4O_6$, 593.2395; found, 593.2988.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-4-(isopentyloxy)benzamide (**81**).* ^{1}H NMR (300 MHz, CD_3CN): δ 8.85 (s, 1H), 8.04 (s, 1H), 7.81 (d, J = 8.7 Hz, 2H), 7.78−7.69 (m, 2H), 7.56 (d, J = 6.4 Hz, 1H), 7.54−7.29 (m, 10H), 7.01 (d, J = 8.6 Hz, 2H), 6.96 (d, J = 8.7 Hz, 2H), 5.62 (d, J = 6.6 Hz, 2H), 5.10 (s, 2H), 4.07 (t, J = 6.7 Hz, 2H), 1.84−1.74 (m, 1H), 1.69−1.50 (m, 2H), 0.97 (s, 3H), 0.95 (s, 3H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{34}H_{36}N_3O_5$, 566.2649; found, 566.2630.

*(S)-3-(2-(4-(Benzyloxy)phenyl)-2-(5-(2,5-dimethylphenoxy)-2,2-dimethylpentanamido)acetamido)benzamide (**82**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.33 (s, 1H), 8.02 (s, 1H), 7.92 (s, 2H), 7.77−7.69 (m, 2H), 7.53 (d, J = 7.9 Hz, 1H), 7.45−7.27 (m, 8H), 7.02−6.93 (m, 3H), 6.68 (s, 1H), 6.61 (d, J = 7.5 Hz, 1H), 5.55 (d, J = 7.3 Hz, 1H), 5.07 (s, 2H), 3.85 (t, J = 5.6 Hz, 2H), 2.23 (s, 3H), 2.06 (s, 3H), 1.77−1.52 (m, 4H), 1.16 (s, 6H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 176.4, 169.3, 167.7, 157.9, 156.5, 138.8, 137.0, 136.0, 135.1, 133.6, 130.2, 130.0, 129.6, 128.6, 128.4, 127.8, 127.6, 127.5, 122.5, 120.5, 118.8, 114.7, 112.1, 69.2, 67.8, 56.7, 41.2, 36.8, 25.2, 25.1, 24.5, 21.0, 15.6. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{37}H_{42}N_3O_5$, 608.3119; found, 608.3115.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)biphenyl-4-carboxamide (**83**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.42 (s, 1H), 8.96 (d, J = 7.3 Hz, 1H), 8.04 (d, J = 8.3 Hz, 2H), 7.93 (s, 2H), 7.82−7.69 (m, 5H), 7.59−7.27 (m, 13H), 7.04 (d, J = 8.4 Hz, 2H), 5.77 (d, J = 7.2 Hz, 1H), 5.11 (s, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 169.2, 167.8, 166.1, 158.1, 142.9, 139.2, 138.9, 137.1, 135.1, 132.6, 129.7, 129.2, 129.0, 128.6, 128.5, 128.4, 128.1, 127.8, 127.6, 126.9, 126.4, 122.2, 121.8, 118.8, 114.7, 69.2, 57.5. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{35}H_{30}N_3O_4$, 556.2231; found, 556.2219.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-4′-chlorobiphenyl-4-carboxamide (**84**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.42 (s, 1H), 8.98 (d, J = 7.2 Hz, 1H), 8.04 (d, J = 7.7 Hz, 2H), 7.93 (s, 2H), 7.85−7.70 (m, 5H), 7.59−7.47 (m, 5H), 7.46−7.27 (m, 7H), 7.03 (d, J = 8.4 Hz, 2H), 5.77 (d, J = 7.1 Hz, 1H), 5.11 (s, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 169.2, 167.7, 165.9, 158.0, 141.5, 138.8, 137.9, 137.0, 135.1, 132.91, 132.86, 129.6, 129.2, 128.9, 128.60, 128.56, 128.5, 128.4, 127.8, 127.5, 126.3, 122.1, 121.7, 118.7, 114.7, 69.1, 57.4. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{35}H_{29}ClN_3O_4$, 590.1841; found, 590.1823.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-4-(furan-2-yl)benzamide (**85**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.41 (s, 1H), 8.99−8.85 (m, 1H), 8.12−7.88 (m, 3H), 7.84−7.74 (m, 3H), 7.69 (d, J = 8.2 Hz, 1H), 7.57−7.28 (m, 11H), 7.23 (d, J = 8.2 Hz, 1H), 7.03 (d, J = 8.3 Hz, 2H), 6.69−6.60 (m, 1H), 5.75 (d, J = 5.1 Hz, 1H), 5.11 (s, 2H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{33}H_{28}N_3O_5$, 546.2023; found, 546.2010.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-4-(pyrimidin-5-yl)benzamide (**86**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.43 (s, 1H), 10.40 (s, 1H), 9.28 (s, 2H), 9.06 (d, J = 7.3 Hz, 1H), 8.10 (d, J = 8.4 Hz, 2H), 8.06 (s, 1H), 7.94 (d, J = 8.2

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Hz, 2H), 7.87−7.72 (m, 2H), 7.52 (d, J = 8.6 Hz, 2H), 7.48−7.30 (m, 8H), 7.04 (d, J = 8.7 Hz, 2H), 5.78 (d, J = 7.2 Hz, 1H), 5.11 (s, 2H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 169.2, 167.8, 165.8, 158.1, 154.9, 150.2, 138.9, 137.0, 136.6, 135.1, 133.9, 129.5, 129.3, 129.2, 128.7, 128.6, 128.4, 127.8, 127.6, 126.7, 124.5, 121.8, 118.8, 114.7, 69.2, 57.5. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{33}H_{28}N_5O_4$, 558.2136; found, 558.2128.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-1-phenylpiperidine-4-carboxamide (**87**).* ^{1}H NMR (300 MHz, acetone-d_6/D_2O): δ 10.82 (s, 1H), 8.41 (s, 1H), 8.00−7.81 (m, 2H), 7.77−7.52 (m, 4H), 7.46 (d, J = 7.0 Hz, 2H), 7.42−7.25 (m, 5H), 7.13−6.94 (m, 4H), 6.82−6.59 (m, 3H), 5.71 (s, 1H), 5.12 (s, 2H), 3.93−2.99 (m, 9H). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{34}H_{35}N_4O_4$, 563.2653; found, 563.2659.

*(S)-N-(1-(4-(Benzyloxy)phenyl)-2-(3-carbamoylphenylamino)-2-oxoethyl)-4-morpholinobenzamide (**88**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.37 (s, 1H), 8.56 (d, J = 7.1 Hz, 1H), 8.04 (s, 1H), 7.92 (s, 2H), 7.85 (d, J = 8.4 Hz, 2H), 7.77 (d, J = 7.9 Hz, 1H), 7.55−7.28 (m, 9H), 7.02 (d, J = 8.3 Hz, 2H), 6.96 (d, J = 8.5 Hz, 2H), 5.73 (d, J = 7.2 Hz, 1H), 5.10 (s, 2H), 3.78−3.69 (m, 4H), 3.28−3.14 (m, 4H). ^{13}C NMR (APT, 75 MHz, DMSO-d_6): δ 169.5, 167.8, 165.9, 158.0, 153.0, 138.9, 137.0, 135.1, 130.0, 129.1, 129.1, 128.6, 128.4, 127.8, 127.6, 123.3, 122.1, 121.8, 118.7, 114.7, 113.2, 69.2, 65.9, 57.2, 47.3. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{33}H_{33}N_4O_5$, 565.2445; found, 565.2451.

Cell Culture. If not stated otherwise, HeLa, Huh-7, and Vero E6 cells were maintained in DMEM supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% heat-inactivated FCS. During infection of Huh-7 cells, DMEM was supplemented with 10 mM HEPES.

DENV2 Reporter Gene Assay (DENV2proHeLa). Stable cells were seeded into 96-well plates with a density of 2 × 10^4 cells per well and treated immediately with inhibitors of the dengue virus protease in a final volume of 100 μL. After incubation for 24 h at 37 °C, the medium was removed, and cells were lysed by adding 25 μL of lysis buffer (Promega) for 15 min at room temperature. Luciferase activity was recorded using a FLUOstar omega plate reader (BMG Labtech) with injections of 100 μL per well of coelenterazine (2.75 μM in PBS). Luminescence was recorded for 5 s. Each concentration was assayed in triplicates. Percent inhibition was calculated in relation to an untreated control. For EC_{50} and EC_{90} calculations, data were fitted and calculated with Prism 6.01 (GraphPad Software, Inc.) using a 4-parameter nonlinear dose−response curve with background subtraction (wells without cells).

Virus Titer Reduction Assay. Huh-7 cells were seeded into 96-well plates at a density of 1 × 10^4 cells in 50 μL per well and incubated overnight. The next day, cells were infected with DENV serotype 2 for 2 h at an MOI of 1, before medium change and compound addition. Infected cells were then incubated for 48 h in the presence of compounds in triplicate wells. In order to determine the EC_{50}, a range of serial diluted compound concentrations starting at 50 μM was used. After 48 h incubation, supernatants were harvested, and triplicates were pooled. These virus-containing supernatants were used to determine the virus titer (reduction) by a plaque assay, as described elsewhere.[61] Briefly, VeroE6 cells were seeded into 24-well plates at a density of 2.5 × 10^5 cells/well and the next day infected for 1 h with 10-fold serial dilutions of the virus supernatant ranging from 10^{-1} to 10^{-6}. After medium exchange and addition of the plaque medium, the VeroE6 cells were incubated for 7 days. Cells were subsequently fixed with formaldehyde, and plaques were visualized by crystal violet staining. At a suitable dilution, plaques were counted, and the virus titer was calculated and plotted against the respective compound concentration. EC_{50} values were derived from fitting data to a dose−response curve (variable-slope, nonlinear regression model) using Prism 6.01 (GraphPad Software, Inc.).

Cytotoxicity. Cell viability in Huh-7 or Hela cells in the presence of compound dilutions was determined using CellTiter-Blue (Promega) according to the manufacturer's instructions. Plates were prepared in parallel to the cell-based DENV reporter or virus titer reduction assay with analogous treatment. Each concentration was assayed in triplicates.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c02042.

Molecular formula strings and activity data (CSV)

Expression and purification of the viral proteases, inhibitory activity of compounds against isolated viral proteases, characterization of compounds **56** and **85**, HPLC-based DENV and WNV protease assays with compounds **56** and **85**, enzyme kinetic studies: Cheng−Prusoff plots, Abz calibration curve, Michaelis−Menten plots, tryptophan fluorescence quenching assay, inhibitory activity of compounds against thrombin and trypsin, residual plots for DENV-2 titer reduction curves, PAMPA, metabolic stability against liver microsomes, stability against pancreatic enzymes, FRET substrate synthesis, synthesis and analytical data of precursors, synthesis and analytical data of inhibitors, HPLC purity of inhibitors, enantiomeric purity of compounds, and ^{1}H und ^{13}C NMR spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Christian D. Klein − *Medicinal Chemistry, Institute of Pharmacy and Molecular Biotechnology IPMB, Heidelberg University, 69120 Heidelberg, Germany;* orcid.org/0000-0003-3522-9182; Phone: +49 6221 544875; Email: c.klein@uni-heidelberg.de; Fax: +49 6221 546430

Authors

Nikos Kühl − *Medicinal Chemistry, Institute of Pharmacy and Molecular Biotechnology IPMB, Heidelberg University, 69120 Heidelberg, Germany;* orcid.org/0000-0001-9025-7245

Mila M. Leuthold − *Medicinal Chemistry, Institute of Pharmacy and Molecular Biotechnology IPMB, Heidelberg University, 69120 Heidelberg, Germany;* orcid.org/0000-0001-7698-2360

Mira A. M. Behnam − *Medicinal Chemistry, Institute of Pharmacy and Molecular Biotechnology IPMB, Heidelberg University, 69120 Heidelberg, Germany;* orcid.org/0000-0002-5839-7675

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jmedchem.0c02042

Notes

The authors declare the following competing financial interest(s): A patent application which covers the chemical structures and medical application of the compounds described in this work was filed by Heidelberg University (PCT/EP2020/056001).

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■ ABBREVIATIONS USED

Boc, *tert*-butoxycarbonyl; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; COMU, (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate; CTC, 2-chlorotrityl chloride; DCM, dichloromethane; DENV, dengue virus; DMEM, Dulbecco's modified Eagle medium; DMF, dimethylformamide; FCS, fetal bovine serum; Fmoc, fluorenylmethyloxycarbonyl; Fmoc-OSu, 9-fluorenylmethyl *N*-succinimidyl carbonate; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MOI, multiplicity of infection; PBS, phosphate-buffered saline; PHG, phenylglycine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TIPS, triisopropylsilane; TMP, 2,4,6-trimethylpyridine; WNV, West Nile virus

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