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Selective targeting of the α C- and DFG-out pocket in p38 MAPK

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Abstract

The p38 MAPK cascade is a key signaling pathway linked to a multitude of physiological functions and of central importance in inflammatory and autoimmune diseases. Although studied extensively, little is known about how conformation-specific inhibitors alter signaling outcomes. Here, we have explored the highly dynamic back pocket of p38 MAPK with allosteric urea fragments. However, screening against known off-targets showed that these fragments maintained the selectivity issues of their parent compound BIRB-796, while combination with the hinge binding motif of VPC-00628 greatly enhanced inhibitor selectivity. Further efforts focused therefore on the exploration of the α C-out pocket of p38 MAPK, yielding compound **137** as a highly selective type-II inhibitor. Even though **137** is structurally related to a recent p38 type-II chemical probe, SR-318, the data presented here provide valuable insights into back-pocket interactions that are not addressed in SR-318, and it provides an alternative chemical tool with good cellular activity targeting also the p38 back pocket.

Keywords: selective type-II p38 MAPK inhibitor, allosteric BIRB-fragments, folded P-loop, differential scanning fluorimetry (DSF), NanoBRETTM assay.

1. Introduction

The mitogen-activating protein kinases (MAPKs) p38 α and p38 β are major mediators of environmental stress signals and have been associated with inflammatory diseases and cancer. By transcriptionally regulating the production of cytokines such as interferon- γ (INF- γ) and tumor necrosis factor- α (TNF- α), p38 MAPK also plays a key role in autoimmune diseases, such as rheumatoid arthritis, psoriasis, diabetes mellitus and multiple sclerosis [1, 2]. Because of its strong association with disease development, p38 α

emerged as a promising drug target [3-5]. However, to date, no p38 α inhibitor has been approved for treatment.

Many p38 inhibitors have been developed, but review of the recent literature revealed that early inhibitors that lack specificity are most frequently used in functional studies. We have therefore developed highly selective and potent type-I and type-I $\frac{1}{2}$ inhibitors as well as very recently also a type-II inhibitor (SR-318) [6]. The MAPKs p38 α and its closely related isoform p38 β are highly dynamic protein kinases, which have been targeted by type-II inhibitors such as BIRB-796, which has high potency for both isoforms but lacks kinome-wide selectivity. A large fraction of human kinase domains can adopt a type-II, DFG-out conformation [7, 8], but targeting this structural feature alone is unlikely to yield selective inhibitors (Figure 1A) [9-11]. Allosteric fragments inspired by the BIRB-796 back-pocket binding motif have been studied, exploring the DFG-out lower selectivity site as well as the allosteric higher selectivity site tolyl pocket [12-16]. An attractive feature of the BIRB-796 back-pocket binding moieties is that they have been associated with very slow off-rate binding kinetics [9, 17, 18]. However, whether selectivity of these fragments against BIRB-796 off-targets can be improved remains to be shown.

An interesting approach has been developed by Decipera et al. [19], who reported on allosteric urea-based fragments that explore the p38 switch-pocket region, by engaging with the less-conserved residues Arg67, Arg149 and the non-conserved Arg70 residue located in the p38 α C-helix and the HRD-loop region. This study led to the development of type-III inhibitors such as DP802 (Figure 1B) [19-22]. However, most of these inhibitors showed weak potency and poor pharmacological properties.

Figure 1. Plasticity of p38 allosteric pockets adjacent to the ATP site and comparison with the MAPK ERK2. A) Type-II p38 inhibitor BIRB-796 (PDB: 1KV2) targeting the inactive DFG-out conformation and the allosteric upper tollyl pocket. B) Type-III p38 inhibitor DP802 (PDB: 3NNW), interacting with the switch-pocket residues. C) Type-II p38 inhibitor VPC00628 (PDB: 5LAR) inducing a P-loop folded conformation. D) Type-IIB inhibitor SCH772984 (PDB: 4QTA) targeting the allosteric P-loop/ α C pocket in a P-loop folded conformation of ERK2.

Unusual binding modes, targeting non-conserved structural elements in kinases, have been demonstrated to favor selectivity and potency of the corresponding inhibitors. A recently published type-II p38 α MAPK inhibitor VPC-00628 [23] that stabilizes a uniquely folded P-loop conformation in p38 α (PDB: 5LAR, Figure 1C) combined a type-II cyclohexyl DFG-out pocket binding moiety with a novel hinge binding motif. Although it is well known that some kinases, such as ABL, ACK1, AURORA, cMET, FGFR1, MAP4K4 and p38, can adopt a folded (inactive) P-loop conformation [24], the enlarged allosteric pocket (P-loop/ α C pocket), created by the movement of the P-loop, has been targeted in the MAPK family only by the ERK1/2 inhibitor SCH772984 (PDB: 4QTA, Figure 1D). Targeting this P-loop/ α C pocket was associated with high inhibitor selectivity and slow dissociation (off-) rates [25]. Interestingly, although SCH772984 did not inhibit p38 upstream kinases, this inhibitor abrogated phosphorylation by MEK1/2 on the ERK activating residues Thr202/Tyr204, as well as on Thr185/Thr187. Therefore, SCH772984 inhibited ERK activation by MEK as well as phosphorylation of ERK substrates. Thus, a synergistic inhibitory effect on Raf-MEK-ERK signaling was observed due to the large structural changes in the N-lobe normally serving as binding sites for MEKs.[26-28] A distinct conformation-selective inhibitory effect on p38 and the MAPK signaling cascade was described by Hari et al. in 2014 [29] using canonical type-I and type-II inhibitors. Interestingly, this study also demonstrated different effects on kinase dephosphorylation by the inactivating phosphatase with dual specificity, DUSP10. In addition, Suvillan et al. reported that inhibitors targeting DFG-out pocket selectivity sites, such as BIRB-796, concurrently inhibited p38 activation and phosphorylation by MEK6, with K_D values in the low nanomolar range, without directly affecting MEK6 activity [18]. These reports make a compelling case that conformation-selective kinase inhibitors may have very different effects on cellular signaling and as a consequence on phenotypic responses.

Here, we focused on the exploration of the highly dynamic allosteric back pocket of p38 α/β . In order to study the effect of diverse decorations targeting the α C-out and DFG-out pocket on ligand selectivity, we

analyzed selectivity of allosteric pyrazole urea fragments on known BIRB-796 off-targets using differential scanning fluorimetry (DSF). These data showed that off-target activity followed closely p38 α / β activity, suggesting that BIRB-796 off-targets remain a liability of its pyrazole urea back-pocket moiety. However, significant selectivity improvement was achieved after fusing the allosteric portion of BIRB-796 with the hinge-binding motif of SR-318, a p38 α / β chemical probe that we recently published that lacks extensive back-pocket interactions apart from the DFG-out binding cyclohexyl moiety. Using SR-318, we explored back-pocket modifications yielding **137**, a potent and selective p38 α / β inhibitor representing an alternative type-II chemical probe targeting p38 α / β .

2. Chemistry

The synthesis of the BIRB-796 fragment library compounds **18-57** was performed in a two-step reaction sequence (Scheme 1). For the preparation of the pyrazole core, heterocycle β -ketonitrile was reacted with a corresponding hydrazine or hydrazine hydrochloride derivative under acidic conditions in ethanol. To maximize the efficiency of the approach, three different microwave-assisted and non-assisted methods (A, B, C) were established and successfully applied [30-34]. The resulting aminopyrazole compounds **1-17** were subsequently reacted with aryl isocyanates in methylene chloride to obtain the final urea compounds **18-57** in low to high yields [30, 35].

Scheme 1. Two-step route for the preparation of urea derivatives **18-57**. Reagents and conditions: (a) hydrazine, toluene/AcOH (5:1), 120 °C (100 W), MW; (b) hydrazine hydrochloride, HCl (cat.), EtOH,

130 °C (200 W), MW; (c) hydrazine hydrochloride, HCl (cat.), EtOH, reflux, 43-95%; (d) isocyanate, CH₂Cl₂, rt, 8-89%; R¹: *tert*-butyl, phenyl, cyclopropyl, CF₃; R² = phenyl, 4-tolyl, *tert*-butyl, methyl, 4-X-phenyl (X = NO₂, Br, F, CF₃, OCF₃, CN, SO₂CH₃); R³ = 2-naphthyl, 1-naphthyl, 2-tolyl, *m*-xylol, 3-methoxyphenyl, *O*-tosyl 3-F-2-tolyl, 3,4-diF-phenyl, 3,5-diCF₃-phenyl, 3,5-diF-phenyl, 4-Y-phenyl (Y = Me, OEt, F, Cl, *O*-benzyl).

For the synthesis of 1-(4-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-5-amine (**61**), a one-pot synthesis with ethyl 2,2,2-trifluoroacetate (**58**) as starting material was used, which was reacted with acetonitrile and potassium *tert*-butoxide in tetrahydrofuran (Scheme 2). The 4,4,4-trifluoro-3-oxobutanenitrile (**59**) produced *in situ* was then heated with (4-nitrophenyl)hydrazine hydrochloride (**60**) in hydrochloric acid and ethanol without further purification to obtain **61** in a yield of 46%.

Scheme 2. One-pot synthesis of compound **61**. Reagents and conditions: (a) Acetonitrile, KO^tBu, THF, rt; (b) HCl, EtOH, reflux, 46%.

Acetanilide analogues **63-65** were synthesized from 1-(3-(*tert*-butyl)-1-(4-nitrophenyl)-1*H*-pyrazol-5-yl)-3-(4-chlorophenyl)urea (**49**) using an Fe-catalyzed Béchamp reduction under mild conditions to obtain compound **62** (Scheme 3). Amide functionalities were then introduced either by activating the carboxylic

acid with HATU, DIPEA in DMF or by a Schotten-Baumann reaction with the corresponding acid chloride or sulfonyl chloride under basic conditions using aniline **62**.

Scheme 3. Synthesis of amino-functionalized compounds **63-65**. Reagents and conditions: (a) Fe, NH₄Cl, EtOH/H₂O (4:1), 70 °C, 75%; (b) HATU, DIPEA, DMF, rt; (c) TEA, THF, 0 °C → rt, 29-84%; R¹ = cyclopropanecarboxamide, *iso*-butylamide, methanesulfonamide.

A convergent synthetic approach was used for synthesizing VPC-00628 derivatives to explore the allosteric back pocket. In the first part of our convergent synthetic route, (*E*)-ethyl 2-cyano-3-ethoxyacrylate (**66**) was reacted with phenylhydrazine to obtain 5-aminopyrazole **67**, which was further treated with an aqueous sodium hydroxide solution to hydrolyze the ester [6]. The acid function of compound **68** was then activated with EDC-HCl/HOBt and reacted with methyl 4-(aminomethyl)benzoate hydrochloride to give the corresponding amide **69**. The latter was then hydrolyzed to the benzoic acid **70** [6]. In the second part, compounds **72-99**, **102** and **105** were synthesized by an amide coupling reaction using the *N*-protected aminoacid **71** as starting material. The subsequent deprotection of the amine functionality under basic conditions led to compounds **106-135**. Using this method, different amide *N*-functionalities were introduced including linear, branched, aliphatic, aromatic and heteroaromatic substituents. In the last step, the amines **106-135** were each coupled with acid **70** using HATU to obtain the final products **136-166** (Scheme 4).

Scheme 4. Convergent synthetic route for the back-pocket optimization, final compounds **136-166**. Reagents and conditions: (a) Phenylhydrazine, EtOH, reflux, 85%; (b) MeOH/THF, NaOH aq., reflux, 98%; (c) Methyl 4-(aminomethyl)benzoate hydrochloride, HOBt, EDC-HCl, DIPEA, DMF, rt, 90%; (d) LiOH, THF/H₂O, rt, 97%; (e) Fmoc-homocyclohexyl-L-alanine, R¹R²NH, HATU, DIPEA, DMF, rt, 48-96%; (f) Piperidine, DMF, rt, quant.; (g) HATU, DIPEA, DMF, rt, 18-96%; R¹ = C (aliphatic, aromatic) and R² = C (aliphatic) or H.

3. Results and Discussion

Kinase conformations are highly dynamic, allowing precise modulation of signaling cascades by catalytic and non-catalytic mechanisms. Allosteric pockets have been discovered coincidentally by trapping less conserved conformational states with small molecules, thereby providing insights into the conformational space available for ligand binding. Here we used p38 MAPK as a well-established model system for which

a large diversity of allosteric and ATP-competitive inhibitors have been developed to study allosteric back-pocket interaction, including the DFG-out and the P-loop/ α C pocket. Allosteric urea-based fragments derived from type-II inhibitor BIRB-796 were synthesized, and their selectivity profile against known BIRB-796 off-targets was evaluated. Some of these allosteric fragments have been studied before; however, their selectivity profiles against BIRB-796 off-targets have not been determined [9, 12, 14-16].

Using DSF, we monitored the activity of a set of 47 kinases, including a selection of BIRB-796 off-targets described by Karaman and Davis et al. ([Supplement Table 1](#)) [11, 36]. In our first synthesized and investigated library, we focused on the modification of the active-site directed aryl urea moiety, with invariant allosteric pyrazole decoration. Keeping each 3,5-di-fluoro-, 3,5-di- CF_3 - and 4-chloro-benzyl decoration constant, we then varied the allosteric upper selectivity site tolyl pocket and BIRB-796 DFG-out *tert*-butyl pocket interactions (compounds **18-48**). During this screen, consistently, highest activity was found for kinases p38 α , p38 β , BRAF, AURKB, SLK, STK10 and EPHA2 (Table 1).

Table 1. Initial BIRB-796 derived urea compound library investigated with DSF assays for off-target inhibition.

No.	Structure	ΔT_m [°C] $\pm$ mean						
		p38 α	p38 β	BRAF	AURKB	SLK	STK10	EPHA2
BIRB-796		19.8 $\pm$ 0.1	19.9 $\pm$ 0.0	22.1 $\pm$ 0.1	4.0 $\pm$ 0.1	3.6 $\pm$ 0.6	10.0 $\pm$ 0.2	8.0 $\pm$ 0.0
18		13.1 $\pm$ 0.1	14.3 $\pm$ 0.5	1.7 $\pm$ 0.1	-3.0 $\pm$ 7.5	2.7 $\pm$ 0.8	1.1 $\pm$ 0.2	2.9 $\pm$ 0.1
19		10.3 $\pm$ 1.0	10.8 $\pm$ 0.0	8.9 $\pm$ 0.3	2.7 $\pm$ 0.7	7.1 $\pm$ 0.4	8.6 $\pm$ 0.1	1.2 $\pm$ 0.1

20		5.3 ± 1.2	5.3 ± 0.8	1.5 ± 0.1	4.3 ± 0.1	1.8 ± 0.9	0.8 ± 0.0	1.6 ± 0.0
21		4.7 ± 0.8	7.0 ± 2.1	3.1 ± 0.0	3.5 ± 0.2	1.1 ± 0.3	0.8 ± 0.0	1.0 ± 0.0
22		8.9 ± 0.3	8.9 ± 0.7	10.1 ± 0.5	4.0 ± 0.0	7.4 ± 0.0	6.5 ± 0.6	2.2 ± 0.0
23		0.2 ± 0.0	0.0 ± 0.1	0.6 ± 0.0	4.1 ± 0.1	0.0 ± 0.0	-0.5 ± 0.2	0.8 ± 0.1
24		1.5 ± 0.4	1.5 ± 0.1	3.0 ± 0.2	3.9 ± 0.1	0.5 ± 0.0	0.4 ± 0.2	1.5 ± 0.0
25		4.8 ± 1.4	4.5 ± 0.2	2.1 ± 0.2	4.0 ± 0.0	2.4 ± 0.8	-0.3 ± 0.2	0.7 ± 0.0
26		9.6 ± 0.4	12.6 ± 0.6	6.0 ± 0.4	4.2 ± 0.1	6.0 ± 0.0	4.1 ± 0.0	1.6 ± 0.0
27		10.2 ± 0.3	9.3 ± 0.2	7.1 ± 0.0	2.3 ± 0.2	5.5 ± 0.6	3.4 ± 0.2	2.2 ± 0.2
28		10.6 ± 0.3	10.8 ± 0.6	9.7 ± 0.2	4.4 ± 0.2	7.7 ± 0.1	7.2 ± 0.2	1.6 ± 0.1
29		-0.4 ± 0.1	-1.3 ± 0.1	0.6 ± 0.1	3.0 ± 0.0	-0.3 ± 0.1	-0.9 ± 0.1	0.2 ± 0.0

30		8.6 ± 0.2	9.8 ± 0.1	8.7 ± 0.4	4.0 ± 0.1	3.6 ± 0.6	3.7 ± 0.0	1.8 ± 0.1
31		10.6 ± 0.1	12.3 ± 0.1	14.1 ± 0.3	3.6 ± 0.3	4.6 ± 0.5	4.7 ± 0.3	1.7 ± 0.1
32		0.3 ± 0.1	-0.2 ± 0.1	1.0 ± 0.0	2.4 ± 1.5	0.0 ± 0.2	-0.5 ± 0.1	0.8 ± 0.0
33		1.4 ± 0.1	1.4 ± 0.0	1.0 ± 0.0	3.3 ± 0.4	2.4 ± 0.2	1.1 ± 0.1	0.4 ± 0.1
34		0.2 ± 0.0	-0.1 ± 0.2	0.4 ± 0.0	3.4 ± 0.6	0.5 ± 0.0	-0.6 ± 0.2	0.4 ± 0.1
35		0.3 ± 0.0	-0.2 ± 0.1	0.9 ± 0.0	4.1 ± 0.1	-0.1 ± 0.1	-0.6 ± 0.1	0.7 ± 0.0
36		0.1 ± 0.0	-0.4 ± 0.2	0.6 ± 0.1	4.0 ± 0.8	-0.2 ± 0.1	-0.7 ± 0.1	0.5 ± 0.0
37		0.1 ± 0.0	0.3 ± 0.1	0.0 ± 0.1	3.1 ± 0.5	0.1 ± 0.0	-0.3 ± 0.0	0.3 ± 0.0
38		2.2 ± 0.1	2.5 ± 0.3	1.4 ± 0.0	4.1 ± 0.0	1.9 ± 0.3	2.6 ± 0.1	0.9 ± 0.0
39		0.1 ± 0.0	-0.4 ± 0.0	0.6 ± 0.1	3.9 ± 0.3	-0.2 ± 0.0	-0.7 ± 0.1	0.5 ± 0.0
40		7.1 ± 1.0	8.6 ± 0.0	5.4 ± 0.3	2.3 ± 2.3	3.0 ± 0.3	2.5 ± 0.3	2.7 ± 0.2

41		7.0 ± 0.8	8.7 ± 0.5	3.7 ± 0.1	-0.1 ± 4.5	2.8 ± 0.4	0.6 ± 0.1	1.9 ± 0.1
42		3.8 ± 0.2	3.4 ± 0.3	1.9 ± 0.1	3.4 ± 0.5	1.2 ± 0.1	1.1 ± 0.1	0.4 ± 0.1
43		5.2 ± 0.5	6.3 ± 1.8	3.3 ± 0.0	4.5 ± 0.1	2.7 ± 1.8	1.7 ± 0.1	1.9 ± 0.1
44		5.0 ± 0.8	6.9 ± 4.9	1.3 ± 0.0	4.0 ± 0.1	2.6 ± 0.7	0.6 ± 0.4	0.9 ± 0.0
45		0.1 ± 0.0	0.0 ± 0.1	0.0 ± 0.1	1.6 ± 1.3	0.2 ± 0.1	-0.2 ± 0.2	0.0 ± 0.0
46		1.6 ± 0.0	1.8 ± 0.6	2.0 ± 0.0	4.0 ± 0.2	0.5 ± 0.0	0.2 ± 0.2	1.3 ± 0.0
47		0.1 ± 0.1	-0.2 ± 0.0	0.4 ± 0.1	3.5 ± 0.1	0.8 ± 0.4	-0.7 ± 0.1	0.3 ± 0.0
48		2.8 ± 0.5	3.2 ± 0.3	0.7 ± 0.1	4.2 ± 0.6	2.6 ± 0.1	0.6 ± 0.2	1.1 ± 0.1
control	Staurosporine	0.4 ± 0.1	0.4 ± 0.1	0.8 ± 0.0	9.9 ± 0.2	16.9 ± 0.1	22.9 ± 0.3	7.0 ± 0.1

ΔT_m average derived from two replicates at a compound concentration of 10 μ M.

This limited screen showed that compounds potently interacting with p38 α / β largely retained activity on kinase targets known to bind to BIRB-796. Examples of such inhibitors are **19**, **22**, **27**, **28** and **31**. Most

compounds harboring a 3,5-di CF₃ decoration, **23** and **32-39**, lost most binding activity for p38, but, interestingly, some retained activity for AURKB, suggesting that these fragments could be developed into chemical probes for this target. From studying 3,5-difluoro decorated compounds **40-48**, hydrophobic DFG-out pocket and tolyl-pocket interactions seem to be important for p38 activity. However, those compounds also had activity on AURKB, SLK and BRAF. Interestingly, allosteric fragments lost activity for EPHA2, emphasizing the importance of an active-site directed moiety for potent inhibition of this receptor tyrosine kinase. A second set of tolyl-pocket modified allosteric compounds, **49-57** and **61-63**, was screened against the kinase panel used before in DSF assay (Table 2).

Table 2. Follow-up BIRB-796 derived urea compound library investigated with DSF assays for off-target inhibition.

No.	Structure	ΔT_m [°C] $\pm$ mean						
		p38 α	p38 β	BRAF	AURKB	SLK	STK10	EPHA2
BIRB-796		19.8 $\pm$ 0.2	19.9 $\pm$ 0.0	22.1 $\pm$ 0.1	4.0 $\pm$ 0.1	3.6 $\pm$ 0.6	10.0 $\pm$ 0.2	8.0 $\pm$ 0.0
49		1.3 $\pm$ 0.1	-0.2 $\pm$ 0.4	0.8 $\pm$ 0.1	3.6 $\pm$ 0.3	0.3 $\pm$ 0.2	0.0 $\pm$ 0.3	0.4 $\pm$ 0.0
62		10.4 $\pm$ 0.9	9.4 $\pm$ 0.5	10.3 $\pm$ 0.5	3.8 $\pm$ 0.4	5.6 $\pm$ 0.8	2.0 $\pm$ 0.2	2.0 $\pm$ 0.2
50		0.9 $\pm$ 0.2	0.3 $\pm$ 0.7	1.1 $\pm$ 0.0	-3.8 $\pm$ 8.0	0.7 $\pm$ 0.8	-0.6 $\pm$ 0.2	0.6 $\pm$ 0.0

51		0.9 ± 0.0	0.6 ± 0.0	1.2 ± 0.0	4.1 ± 0.2	0.2 ± 0.1	0.0 ± 0.1	0.9 ± 0.0
52		3.6 ± 0.1	4.0 ± 0.9	3.3 ± 0.1	4.5 ± 0.1	1.6 ± 0.9	2.2 ± 0.1	0.8 ± 0.0
53		5.6 ± 0.6	7.8 ± 0.3	4.3 ± 0.0	3.7 ± 0.1	5.2 ± 0.9	4.1 ± 0.1	1.2 ± 0.0
63		2.4 ± 0.5	2.6 ± 0.4	5.1 ± 0.2	4.1 ± 0.1	4.1 ± 3.3	3.4 ± 0.1	3.0 ± 0.1
65		6.7 ± 0.0	7.2 ± 0.6	12.2 ± 0.0	3.7 ± 0.0	7.3 ± 0.2	8.9 ± 0.2	2.8 ± 0.1
64		1.5 ± 0.4	1.5 ± 0.4	2.2 ± 0.2	3.9 ± 0.8	6.8 ± 0.3	5.1 ± 0.8	2.8 ± 0.0
54		0.3 ± 0.1	0.3 ± 0.1	-0.2 ± 0.1	2.2 ± 0.4	0.3 ± 0.0	0.0 ± 0.2	0.2 ± 0.0
55		0.2 ± 0.1	-0.3 ± 0.3	0.7 ± 0.0	-2.3 ± 6.8	0.7 ± 0.7	-0.7 ± 0.3	0.5 ± 0.1
56		0.1 ± 0.0	0.6 ± 0.5	0.3 ± 0.3	-2.5 ± 5.8	0.1 ± 0.1	-0.9 ± 0.2	0.2 ± 0.0

57		0.9 ± 0.0	0.6 ± 0.5	0.8 ± 0.0	4.2 ± 0.0	0.6 ± 0.4	0.5 ± 0.2	1.0 ± 0.0
control	Staurosporine	0.4 ± 0.0	0.4 ± 0.1	0.8 ± 0.0	9.9 ± 0.2	16.9 ± 0.1	22.9 ± 0.3	7.0 ± 0.1

ΔT_m average derived from two replicates at a compound concentration of 10 μ M.

This set of inhibitors showed that the tolyl-pocket decoration was important for inhibitor potency on p38 and also most off-targets. It seems that polar donor and acceptor groups present in **53**, **61** and **64** are most potent for p38. For targeting the DFG-out pocket of p38, the bulky *tert*-butyl decoration was preferred, as thermal stabilization was significantly smaller for compounds containing CF₃ and negligible for compounds with a cyclopropyl moiety. However, as observed in the first fragment series, the most potent p38 fragments retained activity against BIRB-796 off-targets such as BRAF, SLK and STK10. Interestingly, some fragments showed selective binding to some off-targets, most notably to SLK and STK10, by compound **63**. Thus, the development of selective inhibitors based on these fragments might be possible.

Recently, we established a series of type-II p38 inhibitors with good p38 selectivity [6]. Some of those inhibitors also showed selectivity for p38 α over the closely related isoform p38 β , in particular when fusing the pyrazole-urea back-pocket decoration of BIRB-796 with the hinge-binding motif of our initial type-II hit VPC00628 [23]. This compound (MCP-081, compound 103 in Röhm et al. 2019 [6]) had an about 30-fold selectivity for p38 α over p38 β (IC_{50} (p38 α /p38 β) = 0.055/1.60 μ M). By screening this type-II inhibitor against our DSF kinase panel, a relatively clean selectivity profile was observed, with off-target activities for AURKB and BRAF only. In order to understand the remarkable α -isoform selectivity of MCP-081 and its allosteric back-pocket interactions, we determined the crystal structure of MCP-081 in complex with p38 α (PDB: 6Y6V). Superposition of this structure with the BIRB-796/p38 α (PDB: 1KV2, Figure 2A) and the VPC-00628/p38 α complex (PDB: 5LAR, not shown) revealed similar back-pocket interactions, while contacts to the hinge region had differing orientations (Figure 2A). These structural

differences may therefore explain the reduced potency of MCP-081 against p38 MAPK that we previously observed. To gain further insights into the structural reasons for the remarkable α -isoform selectivity of MCP-081, we superimposed this structure with the BMS-5c/p38 α complex (PDB: 4KIN, Figure 2B) [37]. Good isoform selectivity for BMS-5c has been reported and rationalized based on a backbone flip between the methionine (Met109) and leucine residue (Leu108) in the hinge region [37] not observed in the MCP-081 structure. We previously speculated that a sequence variation in the back pocket between the p38 α /p38 β isoforms could contribute to isoform selectivity [6]. In position 78 (p38 α numbering), the α -isoform has a methionine, while p38 β has a leucine. However, the similar binding mode described for BIRB-796, which shows no isoform selectivity, makes this difference less likely as a potential explanation for the isoform selectivity of MCP-081. Additional dissimilarities (e.g. differences in the water network) may also contribute to the observed selectivity.

Figure 2. Comparison of the binding modes of MCP-081 (PDB: 6Y6V), BMS-5c (PDB: 4KIN) and BIRB-796 (PDB: 1KV2) in complex with p38 α . A) Superposition of the BIRB-796 fragment with the

VPC-00628 hinge-binding motif in MCP-081 reveals a different binding mode in the active-site region of p38 α . The P-loop in the MCP-081 complex is disordered in the crystal structure due to its high flexibility. B) Superposition of MCP-081 with the α -isoform selective BMS-5c compound. Although both compounds shown exhibited an α -isoform selectivity, flip of the Leu108 backbone was induced by BMS-5c only, thereby questioning a key role of this backbone-flip induction in α -isoform selectivity.

Since structural variations of the BIRB-796 pyrazole-urea motif did not improve selectivity without losing p38 activity, we were interested in whether the VPC-00628 DFG-out back-pocket binding moiety could be optimized by further expansion into the α C-out pocket. Targeting this pocket resulted in high target selectivity for the ERK1/2 inhibitor SCH772984 [25]. In order to explore this pocket, we expanded this lead structure at the terminal primary amide with a diverse set of aliphatic and aromatic building blocks. The obtained compounds **136-166**, were first tested for p38 activity using DSF assays. Inhibition of cellular enzyme activity was then assessed using NanoBRETTM assays in HEK293T [38] cells (Table 3, Table 4). At first, linear and branched aliphatic residues were introduced, resulting in comparable or slightly better protein stabilization, in particular for short hydrophobic aliphatic chains, than with the parent compound VPC-00628 (Table 3). The increase in potency was also seen in the cellular assays performed. Overall, **137** showed the highest potency, with an IC_{50} value of 14.0 ± 6.0 nM in cells and a thermal stabilization, ΔT_m , of 16.4 ± 0.2 °C in DSF assays, and was thus more potent than the lead structure VPC-00628 ($\Delta T_m = 12.9 \pm 1.0$ °C, $IC_{50} = 38.0$ nM). For larger linear and branched decorations, lower ΔT_m values and, as expected, lower potencies were observed. We therefore assumed that the increased lipophilicity and flexibility of longer-chain ligands adversely affected the interaction within the more polar allosteric pocket in p38 α . Interestingly, when one of the methyl groups in the 3-pentyl moiety (**143**) was replaced by a hydroxyl group (**144**), an increase of 2.3 °C in ΔT_m was detected. The hydroxyl group presumably stabilized the ligand inside the binding pocket by interaction with the polar environment of this binding pocket.

Table 3. Activity of p38 back-pocket optimized aliphatic compounds **136-149** investigated by DSF and cellular enzyme activity IC_{50} values determined by NanoBRET™ assay.

Comp.	Structure/R	ΔT_m^a [°C] ± SD p38 α	IC_{50}^b [nM] p38 α	Comp.	Structure/R	ΔT_m^a [°C] ± SD p38 α	IC_{50}^b [nM] p38 α
	BIRB-796	19.8 ^b	7.7	142		14.0 ± 0.4	20.0
VPC-00628 (lead)		12.9 ± 1.0 ^d	38.0	143		10.4 ± 0.4	37.6
136		15.0 ± 0.2 ^c	21.9	144		12.7 ± 0.1	38.2
137		16.4 ± 0.2	14.0 ^c	145		8.6 ± 0.5	93.7
138		13.7 ± 0.5	17.7	146		9.8 ± 0.2	47.0
139		11.3 ± 0.5	33.7	147		13.2 ± 0.3	44.8
140		7.4 ± 0.7	9520	148		12.6 ± 0.3	81.3
141		4.6 ± 0.6	2070	149		5.6 ± 0.3	n.d.

^a ΔT_m average of four measurements; ^b mean of duplicates (n = 2); ^c n = 6; ^d n = 4; n.d. not determined.

The most interesting compounds of this series were co-crystallized with p38 α , and their binding mode was compared with BIRB-796 (Figure 3). The back-pocket modified VPC-00628 derivatives induced a folded conformation of the P-loop not observed in the p38 BIRB-796 complex. All compounds showed a type-II inhibitor binding mode, extending into the DFG-out pocket via their cyclohexyl decoration. As expected, all crystallized compounds formed hinge interactions with the backbone of Met109 and showed a conserved mode of binding. However, for residues targeting the tolyl pocket, the binding mode differed slightly. The tolyl group of BIRB-796 bound near the α C-helix, introduced linear and branched residues pointing towards the solvent-exposed region (Figure 3A). We therefore assumed that the latter observation may explain the difference in potency towards BIRB-796. For cyclic systems that were introduced at this position, interactions closer to the α C helix were observed (Figure 3B).

Figure 3. Binding mode of back-pocket modified lead structures with aliphatic linear, branched and cyclic structures in complex with p38. A) Overlay of the binding modes of linear and branched compounds **137** (lime, PDB: 6YK7), **143** (yellow, PDB: 6Y4U) and **146** (violet, PDB: 6Y4V) with BIRB-796 (pink, PDB: 1KV2). B) Overlay of cyclic compounds **146** (lime, PDB: 6Y4W), **148** (yellow, PDB: 6YJC) and BIRB-

796 (PDB: 1KV2). Hydrogen-bond interactions are highlighted in green. The extended P-loop conformation of p38 in the BIRB-796 complex is highlighted in light pink.

The P-loop/ α C pocket of p38 MAPK harbors an aromatic histidine residue, His64, at a distance of about 14 Å to the terminal amide of the lead structure, and the guanidinium groups of Arg70 and Arg67 are within a distance of 9 Å and 11 Å, respectively. We speculated that the introduction of an aromatic group at the terminal position of our lead structure might form favored π - π stacking and cation- π stacking interactions with these residues. Based on these considerations, various mono- and di-substituted halogenated phenyl residues were introduced to extend the lead structure towards the allosteric back pocket. Subsequently, all compounds were examined again using DSF assays and cellular potencies were determined by NanoBRET™ assays (Table 4).

Table 4. Activity of p38 back-pocket optimized aromatic compounds **150-166** measured by DSF (ΔT_m) and cellular NanoBRET™ assays.

Comp.	Structure/R	ΔT_m^a [°C] ± SD p38 α	IC_{50}^b [nM] p38 α	Comp.	Structure/R	ΔT_m^a [°C] ± SD p38 α	IC_{50}^b [nM] p38 α
150		10.1 ± 0.5	22.1	159		14.7 ± 0.5	21.7
151		7.5 ± 1.1	107	160		13.9 ± 0.4	32.9

152		6.8 ± 0.1	94.4	161		12.6 ± 0.3	25.2
153		7.3 ± 0.2	41.1	162		6.1 ± 2.1	81.3
154		1.9 ± 0.5	1990	163		4.8 ± 0.2	1770
155		2.3 ± 0.3	2830	164		3.1 ± 0.1	2390
156		7.8 ± 1.2 ^c	543	165		3.6 ± 0.1	1040
157		3.5 ± 1.8	287	166		6.0 ± 0.2	462
158		5.1 ± 0.3	144				

^a ΔT_m average of four measurements; ^b IC_{50} values were derived from duplicates (n = 2); ^c n = 6.

Thermal protein stabilization of *para*-substituted halogen compounds (**150-152**) decreased from fluorine to chlorine to bromine. The fluorine-substituted compound **150** seemed therefore most optimal within this series, with a ΔT_m value of 10.1 ± 0.5 °C and an IC_{50} value of 22.1 nM, which was comparable to the lead structure VPC-00628. For 3,5-dihalogen substituted compounds (**153-155**), the ΔT_m values were overall significantly lower, although following the same radius-dependent trend of halogen substitutions. The loss in affinity for the target protein might be due to the increased steric demand and the limited plasticity of the binding pocket. To increase the flexibility of the aforementioned benzylic structures, either a methylene or ethylene bridge between the back-pocket terminal amide and a substituted aromatic or heteroaromatic moiety was introduced (**156-158**). However, a significantly smaller temperature shift was observed

for chlorinated compounds **157** and **158**. Interestingly, for polar picoline compounds (**159-161**) improved activity was observed in ΔT_m assays, which was confirmed by cellular IC_{50} values showing low nanomolar potency. The *para*-substituted picoline derivative (**159**, $\Delta T_m = 14.7 \pm 0.5$ °C, $IC_{50} = 21.7$ nM) seemed to be slightly more potent than the *meta*-substituted (**160**, $\Delta T_m = 13.9 \pm 0.4$ °C, $IC_{50} = 32.9$ nM) and *ortho*-substituted (**161**, $\Delta T_m = 12.6 \pm 0.3$ °C, $IC_{50} = 25.2$ nM) compounds. Less potent binding was detected for the smaller, more electron-rich, five-membered thiophene compound **162**.

An overlay of the crystal structures of the p38 α complexes with chlorine-substituted **150** and **158** with the structure of the BIRB-796 complex illustrated the high plasticity of the allosteric pocket region (Figure 4A). Targeting unique residues such as the non-conserved Arg70 in p38 α MAPK is therefore a challenging task. The more rigid compound **150** bound in closer proximity to the αC helix, and the complex of p38 α with **158** showed that the terminal decoration extended towards the solvent-exposed region. Interestingly, the picoline nitrogen atom of the potent inhibitor **159** sits close to a structural water molecule (4.0 Å) that forms a hydrogen bond with the guanidinium group of Arg70, which is unique to p38 α (Figure 4B), and may form a water-mediated interaction with this arginine in solution. In addition, a structural water molecule in the immediate vicinity bridges the back-pocket carbonyl oxygen of the switch-pocket residue Arg67 with the amide oxygen of **159**.

Figure 4. Binding mode of back-pocket modified VPC-00628 derivatives with aromatic and heteroaromatic structures in complex with p38 α . A) Overlay of the binding modes of aromatic compounds **150** (yellow, PDB: 6ZWP) and **158** (violet, PDB: 6Y4X) with BIRB-796 (pink, PDB: 1KV2). The extended P-loop conformation of p38 α in the BIRB-796 complex is highlighted in light blue. B) Binding mode of picoline derivative **159** (green, PDB: 6ZWR). Selected structural water molecules close to the picoline moiety of **159** are depicted as red spheres. Hydrogen bonds are highlighted with green broken lines. The distance between the picoline ring nitrogen and an Arg70-bound structural water molecule is highlighted in red.

Targeting of the folded P-loop conformation of ERK2 with SCH772984 was possible because this inhibitor harbors a piperazine-phenyl-pyrimidine back-pocket decoration adopting a sharp bend conformation. Because the P-loop/ α C pocket might also be targetable in p38, we synthesized compounds with extensions resembling the SCH772984 back-pocket decoration. To ensure that the inhibitors were flexible enough to adopt a similarly kinked conformation, the piperazine moiety was conserved and decorated with different aromatic and heteroaromatic structures. In comparison with the piperazine derivative **148** ($\Delta T_m = 12.6 \pm 0.3$ °C), substitution of the free NH-hydrogen atom by an aromatic residue led to a sharp decrease in thermal shift values for all compounds that were synthesized (**163-166**). Interestingly, a ΔT_m value of 6.0 ± 0.2 °C was measured for the benzoxazole derivative **166**, whereas replacement of the oxygen atom by a sulfur atom led to an overall loss of stabilization ($\Delta\Delta T_m$ of 2.4 °C; **165**, $\Delta T_m = 3.6 \pm 0.1$ °C). All piperazine-aryl derivatives showed cellular activity on p38 α , with IC_{50} values in the low micromolar range.

The most potent compounds **137** and **159** were chosen for a kinome-wide selectivity screen against a panel of 468 kinases and kinase mutants. As expected from the binding mode observed in the crystal structure, **159** showed a very clean selectivity profile. Main off-targets were DDR1 and EPHA2 (Supple-

mental Table 2). Our most potent compound **137** also showed favorable selectivity, with only seven detected off-targets: DDR1/2, ZAK, ABL1, STK11, MAP3K4 and EPHA1, using a cut-off value of $\leq 35\%$ residual activity at 1 μM compound concentration (Figure 5A). In order to confirm the selectivity screen data, K_D values were determined in dose response for all targets, showing an inhibition $>90\%$ using the *KinomeScan* assay format (Table 5). Interestingly, **137** showed excellent potency for p38 α/β ($K_D = 6.3/20$ nM) with narrow selectivity for the detected off-targets DDR1/2 ($K_D = 31/40$ nM), ZAK ($K_D = 120$ nM) and ABL1 ($K_D = 130$ nM).

Figure 5. Selectivity-creating binding mode. A) Selectivity profiling of **137** against 468 kinases at a compound concentration of 1 μM . Kinase targets with residual activity of $\leq 35\%$ of control without addition of inhibitor are depicted as red dots with radii matching their potency (see insert). B) Co-crystal structure of **137** in complex with p38 α . The P-loop/ αC pocket is highlighted. A 2Fo-Fc electron density map for the ligand **137** is shown at a contour level of 1.5σ (PDB: 6YK7).

We were interested whether the detected off-targets of **137** were also inhibited in the cellular system. Therefore, all off-targets that were detected in the kinome-wide selectivity screen ($\leq 35\%$ residual activity of control) as well as some mutants were tested using NanoBRETTM assays (Table 5). Interestingly, **137**

inhibited p38 α and p38 β *in cellulo* with low nanomolar potencies, whereas only micromolar potencies were determined for the off-targets DDR1, DDR2, ZAK, MAP3K4, FGFR3(G297C) and FLT3(D835Y). No activity ($IC_{50} > 20 \mu\text{M}$) was detected for all other identified off-targets of **137** in cellular NanoBRETTM assays, suggesting that off-target inhibition in cells is not an issue for this compound (Supplemental Figure 1). The differences in activity may be attributed to different K_m values for ATP and discrepancies with inhibiting full-length enzymes used in BRET assays compared with catalytic domains used in the *KinomeScan* and differences in the activation states of the screened kinases.

Table 5. Selectivity profiling and target activity of **137**.

kinome targets	137 % of control at 1 μM	137 DiscoverX K_D [μM]	137 NanoBRET TM $IC_{50} \pm \text{SEM}$ [μM] ^a
FLT3(K663Q)	0	n.d.	>20
FLT3(D835Y)	0	n.d.	>20
DDR1	0.1	0.031	4.19 ± 0.84^b
p38 α	1.5	0.0063	0.014 ± 0.01^c
p38 β	3.4	0.02	0.017 ± 0.001^b
ABL1-nonphosphorylated	8.8	0.13	>20
ZAK	9.4	0.12	1.7
DDR2	9.4	0.04	4.00 ± 0.72^b
MAP3K4	11.0	n.d.	5.80^d
EPHA1	11.0	n.d.	>20
STK11	18.0	n.d.	>20
FGFR3(G697C)	23.0	n.d.	10.0
ABL1(Q252H)-nonphosphorylated	28.0	n.d.	>20

^a IC_{50} values listed are the mean of two experiments (n=2); ^bn = 3; ^cn = 6; ^dn = 1; SEM is given, when n $\geq$ 3; n.d. not determined.

After demonstrating the excellent selectivity of **137** in cells (Figure 5B), the inhibitor was evaluated further by probing its efficiency on endogenous p38 α/β . Human colon adenocarcinoma (HCT-15) cells were

used to study the impact on the endogenous p38 substrate Hsp27, whose phosphorylation is increased in cancer cells such as colorectal cancer and associated with resistance to 5-fluorouracil treatment [39]. After stimulation with the antibiotic anisomycin, analysis by Western blotting revealed that compound **137** showed dose-dependent inhibition of activating phosphorylation of p38 in HCT-15 cells and phosphorylation of its down-stream substrate Hsp27 (Figure 6). However, while effects on p38 activation showed the expected dose response, there was no significant reduction of Hsp27 phosphorylation upon inhibitor treatment, probably due to phosphorylation of this protein by several stress-activated kinases.

Figure 6. Western blot analysis of the effects of inhibitor **137** on p38 α autophosphorylation and Ser82-phosphorylation of Hsp27. HCT-15 cells were treated for 2 h with different concentrations of **137** or vehicle (DMSO) and then stimulated with anisomycin (10 μ g/mL) for 30 min. Vinculin was used as loading control.

As p38 MAPK is related to inflammatory diseases and regulates the expression of pro-inflammatory cytokines, we were further interested in the efficiency of **137** modulating release of TNF- α . Interestingly, the type-II inhibitor **137** showed a significantly stronger inhibitory effect on the TNF- α release than the compound SB203580 [40-42], which inhibits the p38 active conformation, assuming a canonical type-I binding mode. At the highest inhibitor concentration of 10 μ M used in this study, **137** led to an almost

complete inhibition of TNF- α release (92%), and an IC_{50} value of 0.48 ± 0.05 μ M was calculated. In contrast, the type-I inhibitor with an IC_{50} value of 1.36 ± 0.34 μ M and a total inhibition of 83% showed a significantly lower effect on the release of this pro-inflammatory marker.

The metabolic stability of compounds **137**, **150**, **159** and the lead structure VPC-00628 was tested in human liver microsomes (HML). The degradation and metabolite formation of the compounds was followed over 240 min, and each sample was analyzed after 0, 10, 20, 30, 60, 120, 180 and 240 min using LC-MS, ESI(+). The concentration of the protein was standardized at 1 mg/mL for all measurements (Figure 7).

Figure 7. Study on the metabolic stability of selected compounds. A) Metabolic degradation and B) metabolite formation of the lead compound VPC-00628, **137** and **159**, **150** over 240 min in human liver microsomes (HML). Experiments were performed in triplicate. The values represent the mean with standard deviation.

All compounds showed good stability in human liver microsomes. After 240 min, $99.5 \pm 6.3\%$ of compound **159**, $83.5 \pm 8.5\%$ of **150** and $64.0 \pm 2.2\%$ of **137** remained in the media. Compounds **150** and **137** had an increased metabolic stability compared with the lead structure VPC-00628 ($66.9 \pm 2.1\%$ remaining), whereas **159** showed significant stability, with almost no metabolite formation during the 240 min time window that had been investigated.

4. Conclusion

Here, we have developed a series of small molecules for targeting the allosteric α C- and DFG-out pockets in p38 α MAPK. We first synthesized pyrazole urea scaffolds derived from BIRB-796 and tested the activity of those fragments against a comprehensive set of 47 diverse kinases using DSF. The results for this set of compounds were somewhat disappointing, though, as either off-targets described for BIRB-796 were retained or the activity for p38 α/β was significantly reduced. We then revisited our recently published compound MCP-081 that combines the allosteric part of BIRB-796 with the hinge-binding motif of VPC-00628. MCP-081 displayed a clean selectivity profile in our kinase selectivity panel. Intriguingly, a significant selectivity over the p38 β isoform was also observed. As the potency of MCP-081 was reduced compared with VPC-00628 and our recent chemical probe compound SR-318, the allosteric *tert*-butyl pyrazole moiety seemed suboptimal. We synthesized a set of derivatives for targeting the α C-out pocket region, guided by a significant number of crystal structures. Through extension of the terminal amide towards this allosteric site, we developed the potent and selective compound **137**, which showed excellent cellular activity on p38 MAPK in NanoBRETTM assays (IC_{50} [p38 α/β] = 14.0/16.8 nM). In addition, **137** induced an anti-inflammatory response by blocking TNF- α release in whole blood and displayed a high metabolic stability. Taken together, **137** represents a valuable chemical probe for studying

the structural plasticity of p38 MAPK and the resulting diverse phenotypic effects targeting specific conformational states of kinases.

5. Experimental Section

5.1 Chemistry.

All reagents and water-free solvents were purchased from commercial suppliers and were used, if not otherwise stated, without further purification, or solvents were dried by standard procedures. Light petroleum refers to the fraction with bp 40-60 °C. Microwave-assisted reactions were carried out using a CEM DiscoverTM with a CEM Explorer at the given temperature using the instrument's in-built IR temperature measuring device, by modulating the irradiation power (initial power given in parentheses). Column chromatography on silica was carried out on a Biotage Isolera Prime flash purification system for compounds **1-3** and **5-9**. For all other compounds described, column chromatography was performed on silica 60, 0.04-0.63 mm from Machery-Nagel GmbH & Co.KG. Fully characterized compounds were chromatographically homogeneous. Analytical thin layer chromatography was carried out using aluminium-backed plates coated with Merck TLC Silicagel 60 F254. Plates were visualized under UV light (at λ 254 and/or 360 nm), and/or with ninhydrin and potassium permanganate solutions. Melting points were determined for **1-9** and **18-48** using a Stanford Research Systems Optimelt and are uncorrected. IR spectra were recorded in the range from 4000-600 cm^{-1} using a Perkin Trans FT-IR spectrometer. For **10-17**, **49-65**, **67-70**, **72-166** ^1H -NMR and ^{13}C -NMR spectra were recorded either on a Bruker Avance 400, 500 or a Bruker DRX 600 using TMS as internal standard. For **1-9** and **18-48**, NMR spectra were recorded using a Varian VNMRs instrument operating at 400, 500 or 600 MHz for ^1H -NMR and 100 or 126 MHz for ^{13}C -NMR spectroscopy. The chemical shifts (δ) are reported in ppm and are calibrated against the residual proton peak of the deuterated solvent. *J* values were recorded in Hz, and multiplicities were expressed using the

usual conventions. ESI mass spectra (for compounds **1-9**) were obtained using a Bruker Daltonics Apex III, with ESI source Apollo ESI, using MeOH as the spray solvent. For EI mass spectra a Fissons VG Autospec instrument was used at 70 eV and were captured by Dr. Alla K. Abdul-Sada of the University of Sussex Mass Spectrometry Centre. Mass spectrometry (ESI) for **10-17**, **49-65**, **67-70**, **72-166** were measured on a VG Plattform II spectrometer from Fisons. A number of high-resolution mass spectra for **18-48** were obtained courtesy of the EPSRC Mass Spectrometry Service at Swansea University, UK using the ionization methods specified. High-resolution mass spectrometry for **49-57** and **72-166** (FTMS +p MALDI-HRMS) was performed using a MALDI LTQ XL Orbitrap spectrometer from Thermo Scientific. For the final compounds the purity was determined >95%, if not otherwise stated, using HPLC (LC-20A Prominence) Shimadzu. Separations were performed on a C18 column (Luna 10 μ C18 (2) 100 Å; 250 x 4.6 mm) from Phenomenex using the following gradient profile: 0 - 2 min 95% B, 2 - 14 min 95% B, 14 - 21 min 10% B, 21 min 95% B. As solvent A) acetonitrile Ultra MS-Grade was used and as solvent B) MS-Grade H₂O with 0.1% formic acid at a flow rate of 1 mL/min. The detection was carried out with a LCMS-2020 mass spectrometric detector from Shimadzu by a wavelength of 254 and 280 nm respectively.

5.1.1 General procedure A for microwave-assisted synthesis of 5-aminopyrazoles using hydrazine as free base.

A stirred solution of the hydrazine (1 eq) and β -ketonitrile (1 eq) in PhMe/AcOH (5:1, 1-2 mL) was irradiated in a pressure-rated sealed tube at 120 °C for 40 min using a CEM Discover microwave synthesizer by moderating the initial power (100 W). After cooling in a flow of compressed air, the solvent was evaporated *in vacuo*, and the crude product was purified by flash column chromatography on SiO₂.

5.1.2 General procedure B for microwave-assisted synthesis of 5-aminopyrazoles using hydrazine hydrochloride.

A stirred solution of the hydrazine hydrochloride (1 eq), β -ketonitrile (1 eq) and a drop of HCl (conc.) in EtOH (10-20 mL) was irradiated in a pressure-rated sealed tube (35 mL) at 130 °C for 20-30 min using a

CEM Discover microwave synthesizer by moderating the initial power (200 W). After cooling in a flow of compressed air, the mixture was basified to pH 12 by the addition of an aqueous NaOH solution (10%) and extracted with EtOAc (3 x 20 mL). The organic extracts were combined, washed with brine (30 mL), dried over MgSO₄ and the solvent was evaporated *in vacuo*.

5.1.3 General procedure C for synthesis of 5-aminopyrazoles under conductive heating.

A stirred solution of the hydrazine hydrochloride (1 eq), β -ketonitrile (1 eq) and a drop of HCl (conc.) in EtOH (10 mL) was heated at reflux for 18 h. After cooling to rt, the mixture was basified to pH 12 by the addition of an aqueous NaOH solution (10%) (compound 6) or a sat. NaHCO₃ solution (compounds **10-17**) and extracted with EtOAc (3 x 30 mL). The organic extracts were combined, washed with brine (40 mL), dried over Na₂SO₄ and the solvent was evaporated *in vacuo*. Purification by flash column chromatography on SiO₂ or recrystallization from a hexane/EtOAc mixture gave the desired product.

5.1.4 General procedure D for synthesis of *N*-pyrazolyl ureas.

According to a modified procedure[30], the isocyanate (1-1.7 eq) was added to a stirred solution of the 5-aminopyrazole (1 eq) in CH₂Cl₂ (1-3 mL). The mixture was stirred at rt for the given time and then solvent was evaporated *in vacuo* to give the crude product.

5.1.5 General procedure E for amide coupling reaction using HATU for the preparation of intermediate compounds (**72-99**, **102**, **105**).

The carboxylic acid (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 1 eq) and HATU (1.2 eq) were dissolved in DMF (8 mL) and DIPEA (1.2 eq) was added. The solution was stirred at rt for 1.5 h and the corresponding amine (1.2 eq) was added, either pure when liquid or diluted in DMF (4 mL) when solid. The mixture was stirred for 20 h at rt. CH₂Cl₂ or EtOAc (50 mL) was added and the organic phase was washed 4 times with water (50 mL) and dried over MgSO₄. The solvent

was evaporated under reduced pressure and the crude product was purified by column chromatography on silica (eluent: cyclohexane/EtOAc, CH₂Cl₂/MeOH or EtOAc/MeOH) if not stated otherwise.

5.1.6 General procedure for Fmoc-cleavage and the preparation of the amines (106-135).

The amines (**106-135**) used in the amide coupling reaction for general procedure F were freshly prepared directly before coupling to the acid. The corresponding Fmoc protected amine (**72-99**, **102**, **105**, 1.2 eq) was dissolved in DMF (12 mL) and piperidine (20%) was added. The mixture was stirred for 1 h at rt, and CH₂Cl₂ (30 mL) or EtOAc (30 mL) was added. The organic phase was washed 5 times with water (30 mL) and dried over MgSO₄. The solvent was evaporated under reduced pressure, and the crude product was used without further purification.

5.1.7 General procedure F for amide coupling reaction using HATU for the preparation of final compounds (134-164).

The carboxylic acid 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 1 eq) and HATU (1.2 eq) were dissolved in DMF (8 mL) and DIPEA (1.5 eq) was added. The solution was stirred at rt for 1.5 h, and a solution of the corresponding amine (**106-135**, 1.2 eq) in DMF (8 mL) with DIPEA (1.5 eq) was added. The mixture was stirred for 20 h at rt. CH₂Cl₂ or EtOAc (50 mL) was added, and the organic phase was washed 4 times with water (50 mL) and dried over MgSO₄. The solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography on silica (eluent: cyclohexane/EtOAc, CH₂Cl₂/MeOH or EtOAc/MeOH) if not stated otherwise.

5.1.8 5-Amino-3-*tert*-butyl-1-phenyl-1*H*-pyrazole (1), 5-amino-3-*tert*-butyl-1-(*p*-tolyl)-1*H*-pyrazole (2), 5-amino-3-*tert*-butyl-1-methyl-1*H*-pyrazole (3).

Prepared as previously published by Bagley et al. [30].

5.1.9 5-Amino-3-*tert*-butyl-1-(4-fluorophenyl)-1*H*-pyrazole (4).

Prepared according to general procedure B using 4-fluorophenylhydrazine hydrochloride (0.26 g, 1.60 mmol) and 4,4-dimethyl-3-oxopentanenitrile (0.20 g, 1.60 mmol) in EtOH (9 mL) under irradiation for 30 min. Purification by trituration with light petroleum gave 0.32 g (1.37 mmol, 86%) of **4** as a red solid, mp 101.0-105.2 °C; IR: (neat) ν/cm^{-1} 3319, 2969, 1633, 1510, 1216; $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ = 7.55 (2H, dd, $^3J_{\text{HH}} = 9$ Hz, $^3J_{\text{HF}} = 5$ Hz), 7.14 (2H, dd, $^3J_{\text{HH}} = 9$ Hz, $^3J_{\text{HF}} = 9$ Hz), 5.53 (1H, s), 3.65 (2H, bs), 1.32 (9H, s) ppm; $^{13}\text{C-NMR}$ (126 MHz, CDCl_3): δ = 162.4 (C), 161.4 (d, $^1J_{\text{CF}} = 246$ Hz, C), 144.7 (C), 135.2 (C), 125.9 (d, $^3J_{\text{CF}} = 9$ Hz, CH), 116.1 (d, $^2J_{\text{CF}} = 23$ Hz, CH), 87.9 (CH), 32.2 (C), 30.1 (Me) ppm; HRMS (ESI pos.): m/z = 234.1399 $[\text{M}+\text{H}]^+$, calcd. for $[\text{C}_{13}\text{H}_{17}\text{FN}_3]^+ = 234.1401$.

5.1.10 5-Amino-3-*tert*-butyl-1-(4-nitrophenyl)-1*H*-pyrazole (**5**).

Prepared according to general procedure A using 4-nitrophenylhydrazine (0.25 g, 1.60 mmol) and 4,4-dimethyl-3-oxopentanenitrile (0.20 g, 1.60 mmol) in PhMe/AcOH (5:1, 1 mL). Purification by flash column chromatography on SiO_2 , gradient eluting with CH_2Cl_2 /hexane to CH_2Cl_2 , gave 0.21 g (0.81 mmol, 51%) of **5** as a colorless solid, mp 162.1-164.8 °C (lit.[43] mp 172 °C); IR: (neat) ν/cm^{-1} 3395, 2961, 1644, 1592, 1504, 1331, 1241, 1108; $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ = 8.29 (2H, d, $^3J = 9$ Hz), 7.90 (2H, d, $^3J = 9$ Hz), 5.62 (1H, s), 3.82 (2H, bs), 1.31 (9H, s) ppm; $^{13}\text{C-NMR}$ (126 MHz, CDCl_3): δ = 164.0 (C), 145.4 (C), 145.0 (C), 144.8 (C), 124.9 (CH), 122.1 (CH), 90.6 (CH), 32.4 (C), 30.0 (Me) ppm; HRMS (ESI pos.): m/z = 261.1348 $[\text{M}+\text{H}]^+$, calcd. for $[\text{C}_{13}\text{H}_{17}\text{N}_4\text{O}_2]^+ = 261.1346$.

5.1.11 5-Amino-1,3-di-*tert*-butyl-1*H*-pyrazole (**6**).

Prepared according to general procedure C using *tert*-butyl hydrazine hydrochloride (0.66 g, 5.30 mmol) and 4,4-dimethyl-3-oxopentanenitrile (0.50 g, 4.00 mmol) in EtOH (10 mL). Purification by flash column chromatography on SiO_2 , gradient eluting with hexane to hexane/EtOAc (17:3), gave 0.47 g (2.41 mmol, 60%) of **6** as a brown solid, mp 68.7-70.4 °C (lit.[44] mp 67-69 °C); IR: (neat) ν/cm^{-1} 3355, 2962, 1629, 1544, 1359, 1232; $^1\text{H-NMR}$ (500 MHz, DMSO-d_6): δ = 5.24 (1H, s), 4.60 (2H, bs), 1.49 (9H, s), 1.14 (9H, s) ppm; $^{13}\text{C-NMR}$ (126 MHz, DMSO-d_6): δ = 155.9 (C), 146.1 (C), 88.2 (CH), 56.9 (C), 31.5 (C),

30.4 (Me), 29.0 (Me) ppm; MS (EI pos.): m/z (%) = 195.2 (35) ($[M]^+$, calcd. 195.2), 124.2 (100) ($[M_{fr.}]^+$, calcd. 124.2); HRMS (ESI pos.): m/z = 196.1800 $[M+H]^+$, calcd. for $[C_{11}H_{22}N_3]^+ = 196.1808$.

5.1.12 5-Amino-3-*tert*-butyl-1-(4-bromophenyl)-1*H*-pyrazole (7).

Prepared according to general procedure B using 4-bromophenyldiazine hydrochloride (0.35 g, 1.60 mmol) and 4,4-dimethyl-3-oxopentenenitrile (0.20 g, 1.60 mmol) in EtOH (8 mL) under irradiation for 25 min. Purification by flash column chromatography on SiO₂, gradient eluting with hexane to hexane/EtOAc (3:1), gave 0.27 g (0.92 mmol, 58%) of **7** as a brown solid, mp 133.7-136.4 °C; IR: (neat) ν/cm^{-1} 3427, 2957, 1635, 1557, 1500, 1244, 1065; ¹H-NMR (500 MHz, DMSO-*d*₆): δ = 7.62 (2H, d, ³*J* = 9 Hz), 7.56 (2H, d, ³*J* = 9 Hz), 5.40 (1H, s), 5.25 (2H, bs), 1.21 (9H, s) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆): δ = 161.2 (C), 147.2 (C), 138.9 (C), 131.7 (CH), 124.0 (CH), 117.6 (C), 87.4 (CH), 31.7 (C), 30.0 (Me) ppm; MS (EI pos.): m/z (%) = 293.1 (48) ($[M]^+$, calcd. 293.2), 278.6 (100) ($[M_{fr.}]^+$, calcd. 278.6); HRMS (ESI pos.): m/z = 294.0601 $[M+H]^+$, calcd. for $[C_{13}H_{17}BrN_3]^+ = 294.0600$.

5.1.13 5-Amino-1-methyl-3-phenyl-1*H*-pyrazole (8), 5-amino-1,3-diphenyl-1*H*-pyrazole (9).

Prepared as previously published by Bagley et al. [30].

5.1.14 3-(*tert*-Butyl)-1-(4-nitrophenyl)-1*H*-pyrazol-5-amine (10).

Prepared as previously published by Zhu et al. [31].

5.1.15 3-(*tert*-Butyl)-1-(4-(trifluoromethyl)phenyl)-1*H*-pyrazol-5-amine (11).

Prepared as previously published by Li et al. [32].

5.1.16 3-(*tert*-Butyl)-1-(4-(trifluoromethoxy)phenyl)-1*H*-pyrazol-5-amine (12).

Prepared as previously published by King-Underwood et al. [33].

5.1.17 4-(5-Amino-3-(*tert*-butyl)-1*H*-pyrazol-1-yl)benzonitrile (13).

Prepared as previously published by Springer et al. [34].

5.1.18 3-(*tert*-Butyl)-1-(4-(methylsulfonyl)phenyl)-1*H*-pyrazol-5-amine (14).

Prepared according to general procedure C using (4-(methylsulfonyl)phenyl)hydrazine hydrochloride (1.00 g, 4.49 mmol), 4,4-dimethyl-3-oxopentanenitrile (738 mg, 4.49 mmol) and HCl (conc., 2 mL) in EtOH (30 mL), which was heated at reflux for 20 h. The crude product was recrystallized from a hexane/EtOAc mixture (1:1) to yield 907 mg (3.09 mmol, 69%) of **14** as a yellowish solid. TLC: R_F = 0.75 (SiO₂, hexane/EtOAc 1:3); ¹H-NMR (400 MHz, DMSO-*d*₆): δ = 7.98 (d, ³*J* = 8.9 Hz, 2H), 7.90 (d, ³*J* = 8.9 Hz, 2H), 5.52–5.43 (m, 3H), 1.23 (s, 9H) ppm; ¹³C-NMR (101 MHz, DMSO-*d*₆): δ = 162.2, 148.0, 143.7, 136.6, 128.1, 121.6, 88.2, 43.7, 31.9, 30.0 ppm; MS (ESI pos.): *m/z* (%) = 294.2 (100) ([M+H]⁺, calcd. 294.1); 238.1 (25) ([M-SO₂CH₃+Na]⁺, calcd. 185.1).

5.1.19 3-Cyclopropyl-1-(4-(trifluoromethoxy)phenyl)-1*H*-pyrazol-5-amine (15).

Prepared according to general procedure C using (4-(trifluoromethoxy)phenyl)hydrazine hydrochloride (1.00 g, 4.37 mmol), 3-cyclopropyl-3-oxopropanenitrile (643 mg, 4.37 mmol) and HCl (conc., 2 mL) in EtOH (abs., 20 mL), which was heated at reflux for 20 h. The crude product was recrystallized from a cyclohexane/EtOAc mixture (1:1) to yield 956 mg (3.37 mmol, 77%) of **15** as a brownish solid. TLC: R_F = 0.76 (SiO₂, cyclohexane/EtOAc 1:1); ¹H-NMR (500 MHz, DMSO-*d*₆): δ = 7.69 (d, ³*J* = 9.0 Hz, 2H), 7.43 (d, ³*J* = 9.0 Hz, 2H), 5.35 (br s, 2H), 5.20 (s, 1H), 1.79–1.72 (m, 1H), 0.85–0.78 (m, 2H), 0.64–0.58 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆): δ = 154.9, 147.7, 145.5 (2x), 138.6, 123.7, 121.8, 120.2 (¹*J*_{CF} = 256.1 Hz), 87.0, 9.4, 7.5 ppm; MS (ESI pos.): *m/z* (%) = 284.0 (100) ([M+H]⁺, calcd. 284.3).

5.1.20 4-(5-Amino-3-cyclopropyl-1*H*-pyrazol-1-yl)benzonitrile (16).

Prepared according to general procedure C using 4-hydrazinylbenzonitrile hydrochloride (1.00 g, 5.90 mmol), 3-cyclopropyl-3-oxopropanenitrile (643 mg, 5.90 mmol) and HCl (conc., 2 mL) in EtOH

(20 mL) which was heated at reflux for 20 h. The crude product was recrystallized from a cyclohexane/EtOAc mixture (1:1) to yield 939 mg (4.19 mmol, 71%) of **16** as a maroon solid. TLC: R_F = 0.73 (SiO₂, cyclohexane/EtOAc 1:1); ¹H-NMR (500 MHz, DMSO-d₆): δ = 7.93 (d, ³ J = 8.7 Hz, 2H), 7.83 (d, ³ J = 8.7 Hz, 2H), 5.97 (br s, 2H), 5.33 (s, 1H), 1.85–1.78 (m, 1H), 0.92–0.87 (m, 2H), 0.73–0.67 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-d₆): δ = 156.1, 149.0, 141.9, 133.5, 122.5, 118.7, 107.9, 88.0, 9.0, 8.0 ppm; MS (ESI pos.): m/z (%) = 225.1 (100) ([M+H]⁺, calcd. 225.1); 272.1 (14) ([M+EtOH-H]⁺, calcd. 271.2).

5.1.21 3-Cyclopropyl-1-(4-nitrophenyl)-1H-pyrazol-5-amine (17).

Prepared according to general procedure C using (4-nitrophenyl)hydrazine hydrochloride (1.00 g, 5.27 mmol), 3-cyclopropyl-3-oxopropanenitrile (643 mg, 5.27 mmol) and HCl (conc., 2 mL) in EtOH (20 mL) which was heated at reflux for 20 h. The crude product was recrystallized from a cyclohexane/EtOAc mixture (1:1) to yield 631 mg (2.58 mmol, 49%) of **17**, as an orange solid. TLC: R_F = 0.68 (SiO₂, cyclohexane/EtOAc 1:1); ¹H-NMR (600 MHz, DMSO-d₆): δ = 8.29 (d, ³ J = 9.3 Hz, 2H), 7.92 (d, ³ J = 9.3 Hz, 2H), 5.60 (br s, 2H), 5.28 (s, 1H), 1.82–1.76 (m, 1H), 0.88–0.82 (m, 2H), 0.83–0.68 (m, 2H) ppm; ¹³C-NMR (150 MHz, DMSO-d₆): δ = 156.6, 148.7, 144.9, 143.6, 124.7, 121.1, 88.3, 9.3, 7.6 ppm; MS (ESI pos.): m/z (%) = 245.04 (100) ([M+H]⁺, calcd. 245.1).

5.1.22 N-(3-tert-Butyl-1-phenyl-1H-pyrazol-5-yl)-N'-(naphth-1-yl)urea (18).

Prepared as previously published by Regan et.al. [35].

5.1.23 N-(3-tert-Butyl-1-phenyl-1H-pyrazol-5-yl)-N'-(o-tolyl)urea (19).

Prepared according to general procedure D using o-tolyl isocyanate (0.12 mL, 1.60 mmol) and 5-amino-3-tert-butyl-1-phenyl-1H-pyrazole (**1**, 0.20 g, 0.93 mmol) in CH₂Cl₂ (2 mL) at rt for 20 min. Purification by trituration with light petroleum/EtOAc (1:1) gave 0.17 g (0.49 mmol, 53%) of **19** as a colorless solid, mp 166.6–168.0 °C; IR: (neat) ν/cm^{-1} 3291, 2967, 1643, 1551, 1502, 1033; ¹H-NMR (500 MHz, DMSO-

d₆): δ = 8.71 (1H, s, NH), 8.19 (1H, s, NH), 7.54 (4H), 7.40 (1H, m), 7.14 (2H), 6.96 (1H, t, 3J = 7 Hz), 6.36 (1H, s), 2.17 (3H, s), 1.28 (9H, s) ppm; ^{13}C -NMR (126 MHz, DMSO- d_6): δ = 160.7 (C), 152.0 (C), 138.7 (C), 137.2 (C), 136.9 (C), 130.1 (CH), 129.2 (CH), 128.3 (C), 127.0 (CH), 126.0 (CH), 124.0 (CH), 123.2 (CH), 121.8 (CH), 95.8 (CH), 32.0 (C), 30.1 (Me), 17.8 (Me) ppm; HRMS (EI-TOF pos.): m/z = 349.2032 $[\text{M}+\text{H}]^+$, calcd. for $[\text{C}_{21}\text{H}_{25}\text{N}_4\text{O}]^+ = 349.2028$.

5.1.24 *N*-(3-*tert*-Butyl-1-phenyl-1*H*-pyrazol-5-yl)-*N'*-(naphth-2-yl)urea (**20**).

Prepared according to general procedure D using 2-naphthyl isocyanate (0.16 g, 0.93 mmol) and 5-amino-3-*tert*-butyl-1-phenyl-1*H*-pyrazole (**1**, 0.20 g, 0.92 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum/EtOAc (1:1) gave 0.29 g (0.75 mmol, 81%) of **20** as a colorless solid, mp 200.7-206.3 °C (MeOH); IR: (neat) ν/cm^{-1} 3285, 2958, 1654, 1550, 1500, 1033; ^1H -NMR (500 MHz, DMSO- d_6): δ = 9.22 (1H, s, NH), 8.48 (1H, s, NH), 8.09 (1H, s), 7.86-7.73 (3H), 7.56 (4H), 7.46-7.41 (3H), 7.38-7.32 (1H, m), 6.43 (1H, s), 1.30 (9H, s) ppm; ^{13}C -NMR (126 MHz, DMSO- d_6): δ = 160.7 (C), 151.6 (C), 138.5 (C), 137.1 (C), 136.9 (C), 133.6 (C), 129.2 (CH), 129.1 (C), 128.3 (CH), 127.3 (CH), 127.1 (CH), 126.9 (CH), 126.3 (CH), 124.2 (CH), 119.4 (CH), 113.5 (CH), 95.3 (CH), 31.9 (C), 30.1 (Me) ppm; HRMS (EI-TOF pos.): m/z = 385.2021 $[\text{M}+\text{H}]^+$, calcd. for $[\text{C}_{24}\text{H}_{25}\text{N}_4\text{O}]^+ = 385.2028$.

5.1.25 *N*-(3-*tert*-Butyl-1-phenyl-1*H*-pyrazol-5-yl)-*N'*-(4-ethoxyphenyl)urea (**21**).

Prepared according to general procedure D using 4-ethoxyphenyl isocyanate (0.15 mL, 0.93 mmol) and 5-amino-3-*tert*-butyl-1-phenyl-1*H*-pyrazole (**1**, 0.20 g, 0.93 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum/EtOAc (1:1) gave 0.30 g (0.80 mmol, 86%) of **21** as a colorless solid, mp 215.7-219.3 °C; IR: (neat) ν/cm^{-1} 3291, 2966, 1656, 1543, 1510, 1445, 1245, 1050, 1033; ^1H -NMR (500 MHz, DMSO- d_6): δ = 8.78 (1H, s, NH), 8.27 (1H, s, NH), 7.56-7.50 (4H), 7.41 (1H, m), 7.28 (2H, d, 3J = 9 Hz), 6.83 (2H, d, 3J = 9 Hz), 6.35 (1H, s), 3.96 (2H, t, 3J = 7 Hz), 1.34-1.24 (12H) ppm; ^{13}C -NMR (126 MHz, DMSO- d_6): δ = 160.6 (C), 153.8 (C), 151.7 (C), 138.6 (C), 137.3 (C), 132.2 (C), 129.1 (CH), 127.0 (CH), 124.1 (CH), 119.9 (CH), 114.5 (CH), 95.3 (CH), 63.0 (CH_2), 31.9 (C), 30.1

(Me), 14.6 (Me) ppm; HRMS (EI-TOF pos.): $m/z = 379.2130$ $[M+H]^+$, calcd. for $[C_{22}H_{27}N_4O_2]^+ = 379.2134$.

5.1.26 *N*-(3-*tert*-Butyl-1-phenyl-1*H*-pyrazol-5-yl)-*N'*-(4-fluorophenyl)urea (**22**).

Prepared according to general procedure D using 4-fluorophenyl isocyanate (0.15 mL, 1.30 mmol) and 5-amino-3-*tert*-butyl-1-phenyl-1*H*-pyrazole (**1**, 0.20 g, 0.93 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum/EtOAc (5:1) gave 0.28 g (0.80 mmol, 86%) of **22** as a colorless solid, mp 194.0-201.0 °C (MeOH); IR: (neat) ν/cm^{-1} 3294, 2954, 1661, 1544, 1509, 1251, 1033; 1H -NMR (500 MHz, DMSO- d_6): $\delta = 9.01$ (1H, s, NH), 8.36 (1H, s, NH), 7.53 (4H), 7.41 (3H), 7.10 (2H, dd, $^3J_{HH} = 9$ Hz, $^3J_{HF} = 9$ Hz), 6.36 (1H, s), 1.28 (9H, s); ^{13}C -NMR (126 MHz, DMSO- d_6): $\delta = 160.7$ (C), 157.4 (d, $^1J_{CF} = 238$ Hz, C), 151.7 (C), 138.5 (C), 137.0 (CH), 135.6 (d, $^4J_{CF} = 2$ Hz, C), 129.1 (CH), 127.1 (CH), 124.1 (CH), 119.9 (d, $^3J_{CF} = 8$ Hz, CH), 115.2 (d, $^2J_{CF} = 22$ Hz, CH), 95.6 (CH), 31.9 (C), 30.1 (Me); HRMS (EI-TOF pos.): $m/z = 353.1772$ $[M+H]^+$, calcd. for $[C_{20}H_{22}FN_4O]^+ = 353.1778$.

5.1.27 *N*-[3,5-Bis(trifluoromethyl)phenyl]-*N'*-(3-*tert*-butyl-1-phenyl-1*H*-pyrazol-5-yl)urea (**23**).

Prepared according to general procedure D using 3,5-bis(trifluoromethyl)phenyl isocyanate (0.18 mL, 1.00 mmol) and 5-amino-3-*tert*-butyl-1-phenyl-1*H*-pyrazole (**1**, 0.20 g, 0.93 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum gave 0.39 g (0.83 mmol, 89%) of **23** as a colorless solid, mp 175.7-177.8 °C (MeOH); IR: (neat) ν/cm^{-1} 3287, 2967, 1662, 1549, 1502, 1276, 1129, 1033; 1H -NMR (500 MHz, DMSO- d_6): $\delta = 9.70$ (1H, s, NH), 8.67 (1H, s, NH), 8.07 (2H, s), 7.64 (1H, s), 7.56-7.49 (4H), 7.40 (1H, t, $^3J = 6$ Hz), 6.40 (1H, s), 1.29 (9H, s) ppm; ^{13}C -NMR (126 MHz, DMSO- d_6): $\delta = 160.8$ (C), 151.9 (C), 141.5 (C), 138.5 (C), 136.3 (C), 130.7 (q, $^2J_{CF} = 37$ Hz, CH), 129.2 (CH), 127.2 (CH), 124.0 (CH), 124.0 (q, $^1J_{CF} = 272$ Hz, C), 117.9 (CH), 114.6 (CH), 97.1 (CH), 32.0 (C), 30.1 (Me) ppm; MS (EI pos.): m/z (%) = 471.2 (100) ($[M]^+$, calcd. 471.2); HRMS (EI-TOF pos.): $m/z = 471.1617$ $[M+H]^+$, calcd. for $[C_{22}H_{21}F_6N_4O]^+ = 471.1620$.

5.1.28 *N*-(4-Benzyloxyphenyl)-*N'*-(3-*tert*-butyl-1-phenyl-1*H*-pyrazol-5-yl)urea (**24**).

Prepared according to general procedure D using 4-benzyloxyphenyl isocyanate (0.21 g, 0.94 mmol) and 5-amino-3-*tert*-butyl-1-phenyl-1*H*-pyrazole (**1**, 0.20 g, 0.92 mmol) in CH₂Cl₂ (2 mL) at rt for 20 min. Purification by trituration with light petroleum/EtOAc (5:1) gave 0.33 g (0.75 mmol, 82%) of **24** as a colorless solid, mp 185.6-186.6 °C (MeOH); IR: (neat) ν/cm^{-1} 3297, 2957, 1656, 1542, 1509, 1242, 1214, 1005; ¹H-NMR (500 MHz, DMSO-*d*₆): δ = 8.80 (1H, s, NH), 8.28 (1H, s, NH), 7.54-7.49 (4H), 7.44-7.35 (5H), 7.31 (3H), 6.92 (2H, d, ³*J* = 9 Hz), 5.04 (2H, s), 1.27 (9H, s) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆): δ = 160.7 (C), 153.6 (C), 151.7 (C), 138.6 (C), 137.3 (C), 137.2 (C), 132.6 (C), 129.1 (CH), 128.3 (CH), 127.6 (CH), 127.5 (CH), 127.1 (CH), 124.2 (CH), 119.8 (CH), 115.0 (CH), 95.3 (CH), 69.4 (CH₂), 31.9 (C), 30.1 (Me) ppm; HRMS (EI-TOF pos.): *m/z* = 441.2288 [M+H]⁺, calcd. for [C₂₇H₂₉N₄O₂]⁺ = 441.2291.

5.1.29 *N*-(3,5-Dimethylphenyl)-*N'*-(3-*tert*-butyl-1-phenyl-1*H*-pyrazol-5-yl)urea (**25**).

Prepared according to general procedure D using 3,5-dimethylphenyl isocyanate (0.13 mL, 0.93 mmol) and 5-amino-3-*tert*-butyl-1-phenyl-1*H*-pyrazole (**1**, 0.18 g, 0.84 mmol) in CH₂Cl₂ (2 mL) at rt for 20 min. Purification by trituration with light petroleum/EtOAc (1:1) gave 0.27 g (0.75 mmol, 89%) of **25** as a colorless solid, mp 196.7-199.5 °C (MeOH); IR: (neat) ν/cm^{-1} 3001, 2957, 1657, 1543, 1502, 1372, 1214, 1005; ¹H-NMR (500 MHz, DMSO-*d*₆): δ = 8.84 (1H, s, NH), 8.34 (1H, s, NH), 7.52 (4H), 7.41 (1H, m), 7.03 (2H, s), 6.61 (1H, s), 6.38 (1H, s), 2.21 (6H, s), 1.28 (9H, s) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆): δ = 160.7 (C), 151.4 (C), 139.1 (C), 138.5 (C), 137.7 (C), 137.2 (C), 129.2 (CH), 127.1 (CH), 124.2 (CH), 123.6 (CH), 115.8 (CH), 95.2 (CH), 31.9 (C), 30.1 (Me), 21.0 (Me) ppm; HRMS (EI-TOF pos.): *m/z* = 363.2193 [M+H]⁺, calcd. for [C₂₂H₂₇N₄O]⁺ = 363.2185.

5.1.30 *N*-(3-*tert*-Butyl-1-phenyl-1*H*-pyrazol-5-yl)-*N'*-(5-fluoro-2-methylphenyl)urea (**26**).

Prepared according to general procedure D using 5-fluoro-2-methylphenyl isocyanate (0.12 mL, 0.93 mmol) and 5-amino-3-*tert*-butyl-1-phenyl-1*H*-pyrazole (**1**, 0.20 g, 0.91 mmol) in CH₂Cl₂ (2 mL) at rt for 20 min. Purification by trituration with light petroleum/EtOAc (5:1) gave 0.22 g (0.60 mmol, 66%)

of **26** as a colorless solid, mp 164.9-166.4 °C (MeOH); IR: (neat) ν/cm^{-1} 3284, 2958, 1654, 1546, 1503, 1236, 1004; ^1H -NMR (500 MHz, DMSO- d_6): δ = 8.91 (1H, s, NH), 8.31 (1H, s, NH), 7.73 (1H, dd, $^3J_{\text{HF}}$ = 12 Hz, $^4J_{\text{HH}}$ = 2 Hz), 7.54 (4H), 7.42 (1H, m), 7.17 (1H, dd, $^3J_{\text{HH}}$ = 8 Hz, $^3J_{\text{HF}}$ = 8 Hz), 6.76 (1H, ddd, $^3J_{\text{HH}}$ = 8 Hz, $^3J_{\text{HF}}$ = 8 Hz, $^4J_{\text{HH}}$ = 2 Hz), 6.38 (1H, s), 2.15 (3H, s), 1.28 (9H, s) ppm; ^{13}C -NMR (126 MHz, DMSO- d_6): δ = 161.0 (C), 160.5 (d, $^1J_{\text{CF}}$ = 240 Hz, C), 151.9 (C), 138.7 (C), 138.5 (d, $^3J_{\text{CF}}$ = 11 Hz, C), 137.0 (CH), 131.2 (d, $^3J_{\text{CF}}$ = 10 Hz, CH), 129.4 (CH), 127.3 (CH), 124.2 (CH), 122.9 (d, $^4J_{\text{CF}}$ = 3 Hz, C), 109.0 (d, $^2J_{\text{CF}}$ = 21 Hz, CH), 107.3 (d, $^2J_{\text{CF}}$ = 26 Hz, CH), 96.1 (CH), 32.1 (C), 30.2 (Me), 17.2 (Me) ppm; HRMS (EI-TOF pos.): m/z = 367.1937 $[\text{M}+\text{H}]^+$, calcd. for $[\text{C}_{21}\text{H}_{24}\text{FN}_4\text{O}]^+ = 367.1934$.

5.1.31 *N*-(3-*tert*-Butyl-1-phenyl-1*H*-pyrazol-5-yl)-*N'*-(3,4-difluorophenyl)urea (**27**).

Prepared according to general procedure D using 3,4-difluorophenyl isocyanate (0.11 mL, 0.94 mmol) and 5-amino-3-*tert*-butyl-1-phenyl-1*H*-pyrazole (**1**, 0.20 g, 0.92 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum/EtOAc (5:1) gave 0.23 g (0.62 mmol, 67%) of **27** as a colorless solid, mp 154.1-156.4 °C (MeOH); IR: (neat) ν/cm^{-1} 3332, 2957, 1660, 1571, 1513, 1226, 1136; ^1H -NMR (500 MHz, DMSO- d_6): δ = 9.20 (1H, s, NH), 8.44 (1H, s, NH), 7.61 (1H, ddd, $^3J_{\text{HF}}$ = 13 Hz, $^4J_{\text{HF}}$ = 7 Hz, $^4J_{\text{HH}}$ = 2 Hz), 7.52 (4H), 7.41 (1H, m), 7.31 (1H, app q, 3J = 10 Hz), 7.07 (1H, d, $^3J_{\text{HH}}$ = 9 Hz), 6.37 (1H, s), 1.28 (9H, s) ppm; ^{13}C -NMR (126 MHz, DMSO- d_6): δ = 160.7 (C), 151.6 (C), 149.0 (dd, $^1J_{\text{CF}}$ = 243 Hz, $^2J_{\text{CF}}$ = 13 Hz, C), 144.5 (dd, $^1J_{\text{CF}}$ = 240 Hz, $^2J_{\text{CF}}$ = 12 Hz, C), 138.5 (C), 136.7 (C), 136.4 (dd, $^3J_{\text{CF}}$ = 9 Hz, $^4J_{\text{CF}}$ = 2 Hz, C), 129.2 (CH), 127.1 (CH), 124.1 (CH), 117.3 (d, $^3J_{\text{CF}}$ = 6 Hz, $^4J_{\text{CF}}$ = 3 Hz, CH), 114.3 (dd, $^2J_{\text{CF}}$ = 21 Hz, CH), 107.1 (d, $^2J_{\text{CF}}$ = 22 Hz, CH), 96.0 (CH), 31.9 (C), 30.1 (Me) ppm; HRMS (EI-TOF pos.): m/z = 371.1681 $[\text{M}+\text{H}]^+$, calcd. for $[\text{C}_{20}\text{H}_{21}\text{F}_2\text{N}_4\text{O}]^+ = 371.1683$.

5.1.32 *N*-(3-*tert*-Butyl-1-phenyl-1*H*-pyrazol-5-yl)-*N'*-phenylurea (**28**).

Prepared as previously published by Bagley et al. [30].

5.1.33 *N*-[(3-*tert*-Butyl-1-phenyl-1*H*-pyrazol-5-yl)carbonyl]-4-methylbenzenesulfonamide (**29**).

Prepared according to general procedure D using *p*-tolylsulfonyl isocyanate (0.15 mL, 1.10 mmol) and 5-amino-3-*tert*-butyl-1-phenyl-1*H*-pyrazole (**1**, 0.20 g, 0.91 mmol) in CH₂Cl₂ (2 mL) at rt for 20 min. Purification by trituration with light petroleum/EtOAc (1:1) gave 0.28 g (0.68 mmol, 75%) of **29** as a colorless solid, mp 166.3-170.6 °C; IR: (neat) ν/cm^{-1} 3290, 2957, 1657, 1541, 1502, 1212, 989; ¹H-NMR (500 MHz, DMSO-*d*₆): δ = 11.09 (1H, bs, NH), 8.55 (1H, s, NH), 7.77 (2H, d, ³*J* = 8 Hz), 7.47-7.35 (7H), 6.26 (1H, s), 2.40 (3H, s), 1.23 (9H, s) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆): δ = 160.7 (C), 148.8 (C), 143.9 (C), 138.1 (C), 136.7 (C), 135.3 (C), 129.4 (CH), 129.1 (CH), 127.3 (CH), 127.3 (CH), 124.0 (CH), 96.8 (CH), 32.0 (C), 30.0 (Me), 21.0 (Me) ppm; HRMS (EI-TOF pos.): *m/z* = 413.1655 [M+H]⁺, calcd. for [C₂₁H₂₅N₄O₃S]⁺ = 413.1647.

5.1.34 *N*-(3-*tert*-Butyl-1-phenyl-1*H*-pyrazol-5-yl)-*N'*-(3-methoxyphenyl)urea (**30**).

Prepared according to general procedure D using 3-methoxyphenyl isocyanate (0.13 mL, 1.00 mmol) and 5-amino-3-*tert*-butyl-1-phenyl-1*H*-pyrazole (**1**, 0.20 g, 0.93 mmol) in CH₂Cl₂ (2 mL) at rt for 20 min. Purification by trituration with light petroleum/EtOAc (2:1) gave 0.22 g (0.60 mmol, 65%) of **30** as a colorless solid, mp 177.1-179.2 °C (MeOH); IR: (neat) ν/cm^{-1} 3285, 2958, 1661, 1599, 1549, 1045; ¹H-NMR (500 MHz, DMSO-*d*₆): δ = 9.00 (1H, s, NH), 8.35 (1H, s, NH), 7.56-7.49 (4H), 7.41 (1H, m), 7.14 (2H), 6.88 (1H, d, ³*J* = 8 Hz), 6.55 (1H, dd, ³*J* = 8 Hz, 2), 6.38 (1H, s), 3.71 (3H, s), 1.28 (9H, s) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆): δ = 160.7 (C), 159.6 (C), 151.4 (C), 140.5 (C), 138.5 (C), 137.0 (C), 129.4 (CH), 129.2 (CH), 127.1 (CH), 124.2 (CH), 110.3 (CH), 107.6 (CH), 103.8 (CH), 95.4 (CH), 54.8 (Me), 31.9 (C), 30.1 (Me) ppm; HRMS (EI-TOF pos.): *m/z* = 365.1980 [M+H]⁺, calcd. for [C₂₁H₂₅N₄O₂]⁺ = 365.1978.

5.1.35 *N*-(3-*tert*-Butyl-1-phenyl-1*H*-pyrazol-5-yl)-*N'*-(*p*-tolyl)urea (**31**).

Prepared according to general procedure D using *p*-tolyl isocyanate (0.13 mL, 1.00 mmol) and 5-amino-3-*tert*-butyl-1-phenyl-1*H*-pyrazole (**1**, 0.20 g, 0.92 mmol) in CH₂Cl₂ (2 mL) at rt for 20 min. Purification by trituration with light petroleum/EtOAc (1:1) followed by recrystallization gave 0.20 g (0.57 mmol,

62%) of **31** as a colorless solid, mp 197.8-199.2 °C (MeOH); IR: (neat) ν/cm^{-1} 3267, 2955, 1659, 1538, 1362, 1140; $^1\text{H-NMR}$ (500 MHz, DMSO- d_6): δ = 8.88 (1H, s, NH), 8.32 (1H, s, NH), 7.56-7.50 (4H), 7.41 (1H, m), 7.28 (2H, d, 3J = 8 Hz), 7.06 (2H, d, 3J = 8 Hz), 6.37 (1H, s), 2.23 (3H, s), 1.28 (9H, s) ppm; $^{13}\text{C-NMR}$ (126 MHz, DMSO- d_6): δ = 160.7 (C), 151.5 (C), 138.5 (C), 137.2 (C), 136.7 (C), 130.8 (C), 129.2 (CH), 129.1 (CH), 127.1 (CH), 124.2 (CH), 118.2 (CH), 95.2 (CH), 31.9 (C), 30.1 (Me), 20.1 (Me) ppm; HRMS (EI-TOF pos.): m/z = 349.2041 $[\text{M}+\text{H}]^+$, calcd. for $[\text{C}_{21}\text{H}_{25}\text{N}_4\text{O}]^+ = 349.2028$.

5.1.36 *N*-[3,5-Bis(trifluoromethyl)phenyl]-*N'*-[3-*tert*-butyl-1-(*p*-tolyl)-1*H*-pyrazol-5-yl]urea (**32**).

Prepared according to general procedure D using 3,3-bis(trifluoromethyl)phenyl isocyanate (0.19 g, 0.76 mmol) and 5-amino-3-*tert*-butyl-1-(*p*-tolyl)-1*H*-pyrazole (**2**, 0.21 g, 0.90 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum followed by recrystallization gave 0.16 g (0.33 mmol, 43%) of **32** as colorless crystals, mp 155.3-158.3 °C (MeOH); IR: (neat) ν/cm^{-1} 3322, 2959, 1660, 1561, 1383, 1271, 1172, 1139; $^1\text{H-NMR}$ (500 MHz, DMSO- d_6): δ = 9.70 (1H, s, NH), 8.60 (1H, s, NH), 8.06 (2H, s), 7.64 (1H, s), 7.40 (2H, d, 3J = 8 Hz), 7.32 (2H, d, 3J = 8 Hz), 6.38 (1H, s), 1.28 (9H, s) ppm; $^{13}\text{C-NMR}$ (100 MHz, DMSO- d_6): δ = 160.5 (C), 151.8 (C), 141.5 (C), 136.7 (C), 136.2 (C), 136.0 (C), 130.7 (q, $^2J_{\text{CF}} = 33$ Hz, C), 129.6 (CH), 124.1 (CH), 123.4 (q, $^1J_{\text{CF}} = 273$ Hz, C), 117.9 (q, $^3J_{\text{CF}} = 3$ Hz, CH), 114.6 (CH), 96.5 (CH), 32.0 (C), 30.1 (Me) ppm; MS (EI pos.): m/z (%) = 484.2 (100) ($[\text{M}]^+$, calcd. 484.2); HRMS (ESI pos.): m/z = 485.1749 $[\text{M}+\text{H}]^+$, calcd. for $[\text{C}_{23}\text{H}_{23}\text{F}_6\text{N}_4\text{O}]^+ = 485.1771$.

5.1.37 *N*-[3,5-Bis(trifluoromethyl)phenyl]-*N'*-[3-*tert*-butyl-1-methyl-1*H*-pyrazol-5-yl]urea (**33**).

Prepared according to general procedure D using 3,3-bis(trifluoromethyl)phenyl isocyanate (0.15 mL, 0.87 mmol) and 5-amino-3-*tert*-butyl-1-methyl-1*H*-pyrazole (**3**, 0.13 g, 0.84 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum followed by recrystallization gave 0.10 g (0.25 mmol, 30%) of **33** as colorless crystals, mp 155.7-159.7 °C (MeOH); IR: (neat) ν/cm^{-1} 3307, 2968, 1706, 1573, 1386, 1276, 1166, 1130; $^1\text{H-NMR}$ (500 MHz, DMSO- d_6): δ = 9.61 (1H, s, NH), 8.74 (1H, s, NH), 8.14 (2H, s), 7.64 (1H, s), 6.07 (1H, s), 3.61 (3H, Me), 1.22 (9H, s) ppm; $^{13}\text{C-NMR}$ (100 MHz,

DMSO- d_6): δ = 158.6 (C), 152.1 (C), 141.7 (C), 136.2 (C), 130.7 (q, $^2J_{CF}$ = 33 Hz, C), 123.3 (q, $^1J_{CF}$ = 274 Hz, C), 118.0 (CH), 114.5 (CH), 95.0 (CH), 34.9 (Me), 31.8 (C), 30.3 (Me) ppm; HRMS (ESI pos.): m/z = 409.1454 $[M+H]^+$, calcd. for $[C_{17}H_{19}F_6N_4O]^+$ = 409.1458.

5.1.38 *N*-[3,5-Bis(trifluoromethyl)phenyl]-*N'*-[3-*tert*-butyl-1-(4-fluorophenyl)-1*H*-pyrazol-5-yl]urea (**34**).

Prepared according to general procedure D using 3,3-bis(trifluoromethyl)phenyl isocyanate (0.15 mL, 0.87 mmol) and 5-amino-3-*tert*-butyl-1-(4-fluorophenyl)-1*H*-pyrazole (**4**, 0.19 g, 0.82 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum gave 0.30 g (0.61 mmol, 74%) of **34** as a yellow solid, mp 177.6-184.3 °C; IR: (neat) ν/cm^{-1} 3350, 2962, 1664, 1569, 1510, 1383, 1271, 1140; 1H -NMR (500 MHz, DMSO- d_6): δ = 9.70 (1H, s, NH), 8.66 (1H, s, NH), 8.07 (2H, s), 7.63 (1H, s), 7.57 (2H, dd, $^3J_{HH}$ = 9 Hz, $^4J_{HF}$ = 5 Hz), 7.35 (2H, dd, $^3J_{HH}$ = 9 Hz, $^3J_{HF}$ = 9 Hz), 6.39 (1H, s), 1.29 (9H, s) ppm; ^{13}C -NMR (100 MHz, DMSO- d_6): δ = 160.9 (d, $^1J_{CF}$ = 245 Hz, C), 160.8 (C), 151.9 (C), 141.5 (C), 136.4 (C), 135.0 (d, $^4J_{CF}$ = 3 Hz, C), 130.7 (q, $^2J_{CF}$ = 32 Hz, C), 126.3 (d, $^3J_{CF}$ = 9 Hz, CH), 123.2 (q, $^1J_{CF}$ = 273 Hz, C), 117.9 (q, $^3J_{CF}$ = 3 Hz, CH), 115.9 (d, $^2J_{CF}$ = 23 Hz, CH), 114.6 (CH), 97.2 (CH), 32.0 (C), 30.1 (Me) ppm; MS (EI pos.): m/z (%) = 488.1 (100) ($[M]^+$, calcd. 488.1); HRMS (ESI pos.): m/z = 489.1499 $[M+H]^+$, calcd. for $[C_{22}H_{20}F_7N_4O]^+$ = 489.1520.

5.1.39 *N*-[3,5-Bis(trifluoromethyl)phenyl]-*N'*-[3-*tert*-butyl-1-(4-nitrophenyl)-1*H*-pyrazol-5-yl]urea (**35**).

Prepared according to general procedure D using 3,3-bis(trifluoromethyl)phenyl isocyanate (0.12 g, 0.52 mmol) and 5-amino-3-*tert*-butyl-1-(4-nitrophenyl)-1*H*-pyrazole (**5**, 0.15 g, 0.58 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum followed by recrystallization gave 0.10 g (0.19 mmol, 37%) of **35** as orange crystals, mp 215.1-217.8 °C (MeOH); IR: (neat) ν/cm^{-1} 3350, 2968, 1671, 1572, 1504, 1340, 1272, 1175, 1135; 1H -NMR (500 MHz, DMSO- d_6): δ = 9.79 (1H, s, NH), 8.92 (1H, s, NH), 8.35 (2H, d, 3J = 9 Hz), 8.09 (2H, s), 7.88 (2H, d, 3J = 9 Hz), 7.64 (1H, s), 6.48 (1H, s), 1.31 (9H, s) ppm; ^{13}C -NMR (100 MHz, DMSO- d_6): δ = 162.4 (C), 152.2 (C), 145.0 (C), 143.9 (C), 141.5 (C),

137.1 (C), 130.6 (q, $^2J_{\text{CF}} = 32$ Hz, C), 124.7 (CH), 123.6 (q, $^1J_{\text{CF}} = 274$ Hz, C), 123.0 (CH), 118.1 (q, $^3J_{\text{CF}} = 3$ Hz, CH), 114.7 (CH), 100.3 (CH), 32.2 (C), 29.8 (Me) ppm; MS (EI pos.): m/z (%) = 515.1 (100) ($[\text{M}]^{+}$, calcd. 515.1); HRMS (ESI pos.): $m/z = 516.1442$ $[\text{M}+\text{H}]^{+}$, calcd. for $[\text{C}_{22}\text{H}_{20}\text{F}_6\text{N}_5\text{O}_3]^{+} = 516.1465$.

5.1.40 *N*-[3,5-Bis(trifluoromethyl)phenyl]-*N'*-(1,3-di-*tert*-butyl-1*H*-pyrazol-5-yl)urea (36).

Prepared according to general procedure D using 3,3-bis(trifluoromethyl)phenyl isocyanate (0.11 g, 0.43 mmol) and 5-amino-1,3-di-*tert*-butyl-1*H*-pyrazole (**6**, 95 mg, 0.49 mmol) in CH_2Cl_2 (2 mL) at rt for 30 min. Purification by trituration with light petroleum gave 0.14 g (0.31 mmol, 72%) of **36** as a colorless solid, mp 202.2-203.3 °C (MeOH); IR: (neat) ν/cm^{-1} 3310, 2969, 1655, 1576, 1384, 1272, 1184, 1133; ^1H -NMR (500 MHz, $\text{DMSO}-d_6$): $\delta = 9.64$ (1H, s, NH), 8.20 (1H, s, NH), 8.15 (2H, s), 7.62 (1H, s), 6.05 (1H, s), 1.54 (9H, Me), 1.22 (9H, s) ppm; ^{13}C -NMR (100 MHz, $\text{DMSO}-d_6$): $\delta = 156.6$ (C), 153.0 (C), 141.9 (C), 134.5 (C), 130.6 (q, $^2J_{\text{CF}} = 32$ Hz, C), 123.2 (q, $^1J_{\text{CF}} = 273$ Hz, C), 117.9 (CH), 114.4 (CH), 100.7 (CH), 58.8 (C), 31.8 (C), 30.3 (Me), 29.6 (Me) ppm; MS (EI pos.): m/z (%) = 450.1 (30) ($[\text{M}]^{+}$, calcd. 450.1), 139.2 (100) $[\text{M}_{\text{fr}}]^{+}$, calcd. 139.2); HRMS (ESI pos.): $m/z = 451.1923$ $[\text{M}+\text{H}]^{+}$, calcd. for $[\text{C}_{20}\text{H}_{25}\text{F}_6\text{N}_4\text{O}]^{+} = 451.1927$.

5.1.41 *N*-[3,5-Bis(trifluoromethyl)phenyl]-*N'*-[3-*tert*-butyl-1-(4-bromophenyl)-1*H*-pyrazol-5-yl]urea (37).

Prepared according to general procedure D using 3,3-bis(trifluoromethyl)phenyl isocyanate (0.13 g, 0.50 mmol) and 5-amino-3-*tert*-butyl-1-(4-bromophenyl)-1*H*-pyrazole (**7**, 0.16 g, 0.54 mmol) in CH_2Cl_2 (1 mL) at rt for 20 min. Purification by trituration with light petroleum gave 0.16 g (0.29 mmol, 58%) of **37** as a colorless solid, mp 196.5-198.2 °C (MeOH); IR: (neat) ν/cm^{-1} 3286, 2962, 1664, 1564, 1496, 1272, 1179, **1137**; ^1H -NMR (500 MHz, $\text{DMSO}-d_6$): $\delta = 9.70$ (1H, s, NH), 8.71 (1H, s, NH), 8.07 (2H, s), 7.70 (2H, d, $^3J = 8$ Hz), 7.65 (1H, s), 7.51 (2H, d, $^3J = 8$ Hz), 6.40 (1H, s), 1.29 (9H, s) ppm; ^{13}C -NMR (100 MHz, $\text{DMSO}-d_6$): $\delta = 161.7$ (C), 152.4 (C), 142.0 (C), 138.3 (C), 136.9 (C), 132.6 (CH), 131.2 (q, $^2J_{\text{CF}} = 33$ Hz, C), 126.2 (CH), 124.2 (q, $^1J_{\text{CF}} = 273$ Hz, C), 120.3 (C), 118.5 (q, $^3J_{\text{CF}} = 3$ Hz, CH), 115.1 (CH), 98.4 (CH), 32.5 (C), 30.5 (Me) ppm; MS (EI pos.): m/z (%) = 550.2 (37) ($[\text{M}+\text{H}]^{+}$, calcd. 550.2),

306.4 (100) ($[M_{\text{fr.}}]^+$, calcd. 306.4); HRMS (ESI pos.): m/z = 549.0715 $[M+H]^+$, calcd. for $[C_{22}H_{20}BrF_6N_4O]^+ = 549.0719$.

5.1.42 *N*-[3,5-Bis(trifluoromethyl)phenyl]-*N'*-(1-methyl-3-phenyl-1*H*-pyrazol-5-yl)urea (**38**).

Prepared according to general procedure D using 3,3-bis(trifluoromethyl)phenyl isocyanate (0.18 g, 0.71 mmol) and 5-amino-1-methyl-3-phenyl-1*H*-pyrazole (**8**, 0.15 g, 0.85 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum gave 0.22 g (0.51 mmol, 72%) of **38** as a colorless solid, mp 195.3-198.9 °C (MeOH); IR: (neat) ν/cm^{-1} 3378, 2966, 1674, 1561, 1472, 1276, 1125, 1054; 1H -NMR (500 MHz, DMSO- d_6): δ = 9.66 (1H, s, NH), 8.96 (1H, s, NH), 8.18 (2H, s), 7.77 (2H, d, $^3J = 8$ Hz), 7.67 (1H, s), 7.39 (2H, t, $^3J = 8$ Hz), 7.29 (1H, t, $^3J = 8$ Hz), 6.67 (1H, s), 3.74 (3H, Me) ppm; ^{13}C -NMR (100 MHz, DMSO- d_6): δ = 152.1 (C), 148.1 (C), 141.6 (C), 137.6 (C), 133.4 (C), 130.7 (q, $^2J_{CF} = 33$ Hz, C), 128.5 (CH), 127.3 (CH), 124.7 (CH), 123.0 (q, $^1J_{CF} = 273$ Hz, C), 118.2 (CH), 114.7 (CH), 96.2 (CH), 35.4 (Me) ppm; MS (EI pos.): m/z (%) = 428.1 (49) ($[M]^+$, calcd. 428.1), 173.6 (100) ($[M_{\text{fr.}}]^+$, calcd. 173.6); HRMS (ESI pos.): m/z = 429.1146 $[M+H]^+$, calcd. for $[C_{19}H_{15}F_6N_4O]^+ = 429.1144$.

5.1.43 *N*-[3,5-Bis(trifluoromethyl)phenyl]-*N'*-(1,3-diphenyl-1*H*-pyrazol-5-yl)urea (**39**).

Prepared according to general procedure D using 3,3-bis(trifluoromethyl)phenyl isocyanate (0.19 g, 0.73 mmol) and 5-amino-1,3-diphenyl-1*H*-pyrazole (**9**, 0.20 g, 0.86 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum followed by recrystallization gave 0.23 g (0.47 mmol, 64%) of **39** as colorless crystals, mp 205.7-206.9 °C (MeOH); IR: (neat) ν/cm^{-1} 3306, 2967, 1653, 1590, 1574, 1474, 1274, 1126, 1055; 1H -NMR (500 MHz, DMSO- d_6): δ = 9.72 (1H, s, NH), 8.86 (1H, s, NH), 8.10 (2H, s), 7.88 (2H, d, $^3J = 7$ Hz), 7.65 (3H), 7.58 (2H, t, $^3J = 8$ Hz), 7.49-7.41 (3H), 7.35 (1H, t, $^3J = 7$ Hz), 6.99 (1H, s) ppm; ^{13}C -NMR (100 MHz, DMSO- d_6): δ = 151.9 (C), 150.1 (C), 141.4 (C), 138.3 (C), 137.6 (C), 132.9 (C), 130.7 (q, $^2J_{CF} = 32$ Hz, C), 129.3 (CH), 128.6 (CH), 127.9 (CH), 127.8 (CH), 125.1 (CH), 124.3 (CH), 123.7 (q, $^1J_{CF} = 273$ Hz, C), 118.1 (CH), 114.8 (CH), 98.0 (CH) ppm; MS (EI pos.):

m/z (%) = 490.1 (45) ($[M]^{+}$, calcd. 490.1), 235.3 (100) ($[M_{fr.}]^{+}$, calcd. 235.3); HRMS (ESI pos.): m/z = 491.1288 $[M+H]^{+}$, calcd. for $[C_{24}H_{17}F_6N_4O]^{+}$ = 491.1301.

5.1.44 *N*-(3,5-Difluorophenyl)-*N'*-(3-*tert*-butyl-1-phenyl-1*H*-pyrazol-5-yl)urea (**40**).

Prepared according to general procedure D using difluorophenyl isocyanate (0.12 g, 0.74 mmol) and 5-amino-3-*tert*-butyl-1-phenyl-1*H*-pyrazole (**1**, 0.16 g, 0.74 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum gave 0.17 g (0.46 mmol, 62%) of **40** as a colorless solid, mp 171.0-173.0 °C (MeOH); IR: (neat) ν/cm^{-1} 3322, 2956, 1670, 1554, 1500, 1231, 1117; 1H -NMR (500 MHz, DMSO- d_6): δ = 9.38 (1H, s, NH), 8.54 (1H, s, NH), 7.53 (4H), 7.44-7.36 (1H, m), 7.14 (2H, d, $^3J_{HF}$ = 9 Hz), 6.78 (1H, t, $^3J_{HF}$ = 9 Hz), 6.38 (1H, s), 1.29 (9H, s) ppm; ^{13}C -NMR (100 MHz, DMSO- d_6): δ = 162.5 (dd, $^1J_{CF}$ = 243 Hz, $^3J_{CF}$ = 15 Hz, C), 160.7 (C), 151.5 (C), 142.1 (t, $^3J_{CF}$ = 14 Hz, C), 138.5 (C), 136.5 (C), 129.2 (CH), 127.2 (CH), 124.1 (CH), 100.9 (dd, $^2J_{CF}$ = 21 Hz, $^4J_{CF}$ = 8 Hz, CH), 97.0 (t, $^2J_{CF}$ = 27 Hz, CH), 96.3 (CH), 32.0 (C), 30.1 (Me) ppm; MS (EI pos.): m/z (%) = 370.1 (47) ($[M]^{+}$, calcd. 370.1), 139.2 (100) ($[M_{fr.}]^{+}$, calcd. 139.2); HRMS (EI-TOF pos.): m/z = 371.1673 $[M+H]^{+}$, calcd. for $[C_{20}H_{21}F_2N_4O]^{+}$ = 371.1678.

5.1.45 *N*-(3,5-Difluorophenyl)-*N'*-[3-*tert*-butyl-1-(*p*-tolyl)-1*H*-pyrazol-5-yl]urea (**41**).

Prepared according to general procedure D using difluorophenyl isocyanate (0.11 g, 0.68 mmol) and 5-amino-3-*tert*-butyl-1-(*p*-tolyl)-1*H*-pyrazole (**2**, 0.16 g, 0.70 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum gave 0.17 g (0.44 mmol, 65%) of **41** as a colorless solid, mp 188.9-190.8 °C (MeOH); IR: (neat) ν/cm^{-1} 3375, 2963, 1720, 1619, 1542, 1478, 1196, 1114; 1H -NMR (500 MHz, DMSO- d_6): δ = 9.37 (1H, s, NH), 8.47 (1H, s, NH), 7.38 (2H, d, 3J = 8 Hz), 7.32 (2H, d, 3J = 8 Hz), 7.13 (2H, d, $^3J_{HF}$ = 9 Hz), 6.79 (1H, t, $^3J_{HF}$ = 9 Hz), 6.35 (1H, s), 2.37 (3H, s), 1.27 (9H, s) ppm; ^{13}C -NMR (100 MHz, DMSO- d_6): δ = 162.5 (dd, $^1J_{CF}$ = 243 Hz, $^3J_{CF}$ = 16 Hz, C), 160.5 (C), 151.4 (C), 142.1 (t, $^3J_{CF}$ = 14 Hz, C), 136.8 (C), 136.5 (C), 136.0 (C), 129.6 (CH), 124.2 (CH), 100.9 (dd, $^2J_{CF}$ = 29 Hz, $^4J_{CF}$ = 8 Hz, CH), 97.0 (t, $^2J_{CF}$ = 26 Hz, CH), 95.7 (CH), 32.0 (C), 30.2 (Me), 20.5 (Me) ppm; MS (EI

pos.): m/z (%) = 384.2 (52) ($[M]^{++}$, calcd. 384.2), 214.2 (100) ($[M_{fr.}]^+$, calcd. 214.2); HRMS (ESI pos.): m/z = 385.1829 $[M+H]^+$, calcd. for $[C_{21}H_{23}F_2N_4O]^+ = 385.1834$.

5.1.46 *N*-(3,5-Difluorophenyl)-*N'*-(3-*tert*-butyl-1-methyl-1*H*-pyrazol-5-yl)urea (**42**).

Prepared according to general procedure D using difluorophenyl isocyanate (0.14 g, 0.93 mmol) and 5-amino-3-*tert*-butyl-1-methyl-1*H*-pyrazole (**3**, 0.16 g, 1.00 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum followed by recrystallization gave 0.22 g (0.71 mmol, 76%) of **42** as colorless crystals, mp 189.0-191.1 °C (MeOH); IR: (neat) ν/cm^{-1} 3322, 2964, 1711, 1610, 1558, 1478, 1206, 1112; 1H -NMR (500 MHz, DMSO- d_6): δ = 9.27 (1H, s, NH), 8.61 (1H, s, NH), 7.19 (2H, d, $^3J_{HF}$ = 8 Hz), 6.80 (1H, t, $^3J_{HF}$ = 9 Hz), 6.04 (1H, s), 3.59 (3H, s), 1.21 (9H, s) ppm; ^{13}C -NMR (100 MHz, DMSO- d_6): δ = 162.5 (dd, $^1J_{CF}$ = 243 Hz, $^3J_{CF}$ = 15 Hz, C), 158.5 (C), 151.7 (C), 142.2 (t, $^3J_{CF}$ = 14 Hz, C), 136.4 (C), 101.0 (dd, $^2J_{CF}$ = 29 Hz, $^4J_{CF}$ = 8 Hz, CH), 96.9 (t, $^2J_{CF}$ = 26 Hz, CH), 94.4 (CH), 34.9 (Me), 31.8 (C), 30.3 (Me) ppm; MS (EI pos.): m/z (%) = 308.1 (56) ($[M]^{++}$, calcd. 308.1), 138.6 (100) ($[M_{fr.}]^+$, calcd. 138.6); HRMS (ESI pos.): m/z = 309.1517 $[M+H]^+$, calcd. for $[C_{15}H_{19}F_2N_4O]^+ = 309.1521$.

5.1.47 *N*-(3,5-Difluorophenyl)-*N'*-[3-*tert*-butyl-1-(4-fluorophenyl)-1*H*-pyrazol-5-yl]urea (**43**).

Prepared according to general procedure D using difluorophenyl isocyanate (80 mg, 0.52 mmol) and 5-amino-3-*tert*-butyl-1-(4-fluorophenyl)-1*H*-pyrazole (**4**, 0.13 g, 0.55 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum followed by recrystallization gave 80 mg (0.21 mmol, 40%) **43** as colorless crystals, mp 165.3-169.3 °C (MeOH); IR: (neat) ν/cm^{-1} 2966, 1728, 1612, 1553, 1226, 1113; 1H -NMR (500 MHz, DMSO- d_6): δ = 9.35 (1H, s, NH), 8.52 (1H, s, NH), 7.55 (2H, dd, $^3J_{HH}$ = 8 Hz, $^4J_{HF}$ = 5 Hz), 7.36 (2H, dd, $^3J_{HH}$ = 8 Hz, $^3J_{HF}$ = 8 Hz), 7.13 (2H, d, $^3J_{HF}$ = 8 Hz), 6.79 (1H, t, $^3J_{HF}$ = 9 Hz), 6.36 (1H, s), 1.28 (9H, s) ppm; ^{13}C -NMR (100 MHz, DMSO- d_6): δ = 161.2 (dd, $^1J_{CF}$ = 243 Hz, $^3J_{CF}$ = 16 Hz, C), 160.9 (d, $^1J_{CF}$ = 245 Hz, C), 160.8 (C), 151.5 (C), 142.0 (t, $^3J_{CF}$ = 14 Hz, C), 136.7 (C), 134.9 (d, $^4J_{CF}$ = 3 Hz, C), 126.5 (d, $^3J_{CF}$ = 8 Hz, CH), 116.0 (d, $^2J_{CF}$ = 23 Hz, CH), 100.9 (dd, $^2J_{CF}$ = 21 Hz, $^4J_{CF}$ = 9 Hz, CH), 97.0 (t, $^2J_{CF}$ = 26 Hz, CH), 96.4 (CH), 32.0 (C), 30.1 (Me) ppm; MS (EI pos.): m/z (%)

= 389.1 (72) ($[M+H]^+$, calcd. 389.1), 218.9 (100) ($[M_{fr.}]^+$, calcd. 218.9); HRMS (ESI pos.): m/z = 389.1582 $[M+H]^+$, calcd. for $[C_{20}H_{20}F_3N_4O]^+$ = 389.1584.

5.1.48 *N*-(3,5-Difluorophenyl)-*N'*-[3-*tert*-butyl-1-(4-nitrophenyl)-1*H*-pyrazol-5-yl]urea (**44**).

Prepared according to general procedure D using difluorophenyl isocyanate (150 mg, 0.58 mmol) and 5-amino-3-*tert*-butyl-1-(4-nitrophenyl)-1*H*-pyrazole (**5**, 82 mg, 0.52 mmol) in CH_2Cl_2 (2 mL) at rt for 18 h. Purification by trituration with light petroleum followed by recrystallization gave 54 mg (0.13 mmol, 25%) of **44** as yellow crystals, mp 178.6-182.0 °C (MeOH); IR: (neat) ν/cm^{-1} 3298, 2963, 1762, 1610, 1565, 1503, 1334, 1234, 1116; 1H -NMR (500 MHz, DMSO- d_6): δ = 9.45 (1H, s, NH), 8.78 (1H, s, NH), 8.36 (2H, d, $^3J_{HH}$ = 9 Hz), 7.87 (2H, d, $^3J_{HH}$ = 9 Hz), 7.14 (2H, d, $^3J_{HF}$ = 8 Hz), 6.79 (1H, t, $^3J_{HF}$ = 9 Hz), 6.45 (1H, s), 1.30 (9H, s) ppm; ^{13}C -NMR (100 MHz, DMSO- d_6): δ = 162.5 (dd, $^1J_{CF}$ = 243 Hz, $^3J_{CF}$ = 16 Hz, C), 162.4 (C), 151.8 (C), 145.0 (C), 143.9 (C), 142.0 (t, $^3J_{CF}$ = 14 Hz, C), 137.4 (C), 124.8 (CH), 123.1 (CH), 101.1 (dd, $^2J_{CF}$ = 21 Hz, $^4J_{CF}$ = 9 Hz, CH), 99.5 (CH), 97.4 (d, $^2J_{CF}$ = 27 Hz, CH), 32.2 (C), 29.9 (Me) ppm; MS (EI pos.): m/z (%) = 415.2 (30) ($[M]^+$, calcd. 415.2), 271.3 (100) ($[M_{fr.}]^+$, calcd. 271.3); HRMS (ESI pos.): m/z = 416.1534 $[M+H]^+$, calcd. for $[C_{20}H_{20}F_2N_5O_3]^+$ = 416.1529.

5.1.49 *N*-(3,5-Difluorophenyl)-*N'*-(1,3-di-*tert*-butyl-1*H*-pyrazol-5-yl)urea (**45**).

Prepared according to general procedure D using difluorophenyl isocyanate (0.11 g, 0.68 mmol) and 5-amino-1,3-di-*tert*-butyl-1*H*-pyrazole (**6**, 0.14 g, 0.72 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum followed by recrystallization gave 0.16 g (0.46 mmol, 68%) of **45** as colorless crystals, mp 212.9-214.5 °C (MeOH); IR: (neat) ν/cm^{-1} 3366, 2957, 1663, 1612, 1566, 1478, 1207, 1116; 1H -NMR (500 MHz, DMSO- d_6): δ = 9.31 (1H, s, NH), 8.01 (1H, s, NH), 7.18 (2H, d, $^3J_{HF}$ = 8 Hz), 6.77 (1H, t, $^3J_{HF}$ = 9 Hz), 6.02 (1H, s), 1.53 (9H, s), 1.21 (9H, s) ppm; ^{13}C -NMR (100 MHz, DMSO- d_6): δ = 162.5 (dd, $^1J_{CF}$ = 243 Hz, $^3J_{CF}$ = 15 Hz, C), 156.5 (C), 152.6 (C), 142.5 (t, $^3J_{CF}$ = 15 Hz, C), 134.6 (C), 100.9 (dd, $^2J_{CF}$ = 30 Hz, $^4J_{CF}$ = 9 Hz, CH), 100.4 (CH), 96.7 (t, $^2J_{CF}$ = 27 Hz, CH), 58.7 (C), 31.8 (C),

30.3 (Me), 29.5 (Me) ppm; MS (EI pos.): m/z (%) = 350.2 (39) ($[M]^+$, calcd. 350.2), 139.2 (100) ($[M_{fr.}]^+$, calcd. 139.2); HRMS (ESI pos.): m/z = 351.1987 $[M+H]^+$, calcd. for $[C_{18}H_{25}F_2N_4O]^+ = 351.1991$.

5.1.50 *N*-(3,5-Difluorophenyl)-*N'*-[3-*tert*-butyl-1-(4-bromophenyl)-1*H*-pyrazol-5-yl]urea (**46**).

Prepared according to general procedure D using difluorophenyl isocyanate (0.17 g, 1.00 mmol) and 5-amino-3-*tert*-butyl-1-(4-bromophenyl)-1*H*-pyrazole (**7**, 0.21 g, 0.71 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum followed by recrystallization gave 0.17 mg (0.38 mmol, 54%) of **46** as colorless crystals, mp 158.0-159.4 °C (MeOH); IR: (neat) ν/cm^{-1} 3320, 2964, 1668, 1612, 1552, 1478, 1228, 1116, 670; 1H -NMR (500 MHz, DMSO- d_6): δ = 9.37 (1H, s, NH), 8.58 (1H, s, NH), 7.71 (2H, d, $^3J_{HH} = 9$ Hz), 7.50 (2H, d, $^3J_{HH} = 9$ Hz), 7.14 (2H, d, $^3J_{HF} = 8$ Hz), 6.78 (1H, t, $^3J_{HF} = 9$ Hz), 6.38 (1H, s), 1.28 (9H, s) ppm; ^{13}C -NMR (100 MHz, DMSO- d_6): δ = 163.8 (dd, $^1J_{CF} = 243$ Hz, $^3J_{CF} = 16$ Hz, C), 161.2 (C), 151.6 (C), 142.0 (t, $^3J_{CF} = 14$ Hz, C), 137.8 (C), 136.7 (C), 132.1 (CH), 125.9 (CH), 119.8 (C), 101.0 (dd, $^2J_{CF} = 21$ Hz, $^4J_{CF} = 9$ Hz, CH), 97.0 (2CH), 32.0 (C), 30.0 (Me) ppm; MS (EI pos.): m/z (%) = 449.1 (79) ($[M+H]^+$, calcd. 449.1), 279.4 (100) ($[M_{fr.}]^+$, calcd. 279.4); HRMS (ESI pos.): m/z = 449.0782 $[M+H]^+$, calcd. for $[C_{20}H_{20}BrF_2N_4O]^+ = 449.0783$.

5.1.51 *N*-(3,5-Difluorophenyl)-*N'*-(1-methyl-3-phenyl-1*H*-pyrazol-5-yl)urea (**47**).

Prepared according to general procedure D using difluorophenyl isocyanate (0.10 g, 0.66 mmol) and 5-amino-1-methyl-3-phenyl-1*H*-pyrazole (**8**, 0.13 g, 0.73 mmol) in CH_2Cl_2 (3 mL) at rt for 20 min. Purification by trituration with light petroleum followed by recrystallization gave 0.18 g (0.55 mmol, 83%) of **47** as colorless crystals, mp 205.6-206.7 °C (MeOH); IR: (neat) ν/cm^{-1} 3284, 2973, 1711, 1609, 1558, 1479, 1235, 1114; 1H -NMR (500 MHz, DMSO- d_6): δ = 9.34 (1H, s, NH), 8.82 (1H, s, NH), 7.76 (2H, d, $^3J_{HH} = 8$ Hz), 7.39 (2H, t, $^3J_{HH} = 8$ Hz), 7.28 (1H, t, $^3J_{HH} = 8$ Hz), 7.22 (2H, d, $^3J_{HF} = 9$ Hz), 6.81 (1H, t, $^3J_{HF} = 9$ Hz), 6.64 (1H, s), 3.73 (3H, s) ppm; ^{13}C -NMR (100 MHz, DMSO- d_6): δ = 163.0 (dd, $^1J_{CF} = 243$ Hz, $^3J_{CF} = 15$ Hz, C), 151.7 (C), 148.1 (C), 142.1 (t, $^3J_{CF} = 14$ Hz, C), 137.8 (C), 133.5 (C), 128.5 (CH), 127.3 (CH), 124.7 (CH), 101.1 (dd, $^2J_{CF} = 21$ Hz, $^4J_{CF} = 8$ Hz, CH), 96.8 (t, $^2J_{CF} = 26$ Hz, CH), 95.5

(CH), 35.4 (Me) ppm; MS (EI pos.): m/z (%) = 328.0 (44) ($[M]^+$, calcd. 328.0), 173.5 (100) ($[M_{fr.}]^+$, calcd. 173.5); HRMS (ESI pos.): m/z = 329.1204 $[M+H]^+$, calcd. for $[C_{17}H_{15}F_2N_4O]^+ = 329.1208$.

5.1.52 *N*-(3,5-Difluorophenyl)-*N'*-(1,3-diphenyl-1*H*-pyrazol-5-yl)urea (**48**).

Prepared according to general procedure D using difluorophenyl isocyanate (0.12 g, 0.75 mmol) and 5-amino-1,3-diphenyl-1*H*-pyrazole (**9**, 0.20 g, 0.85 mmol) in CH_2Cl_2 (2 mL) at rt for 20 min. Purification by trituration with light petroleum followed by recrystallization gave 0.21 g (0.54 mmol, 72%) of **48** as colorless crystals, mp 195.4-200.4 °C (MeOH); IR: (neat) ν/cm^{-1} 3324, 2945, 1726, 1615, 1544, 1480, 1194, 1106; 1H -NMR (500 MHz, DMSO- d_6): δ = 9.42 (1H, s, NH), 8.71 (1H, s, NH), 7.86 (2H, d, $^3J_{HH} = 8$ Hz), 7.60 (4H), 7.46 (3H), 7.35 (1H, t, $^3J_{HH} = 8$ Hz), 7.16 (2H, d, $^3J_{HF} = 9$ Hz), 6.95 (1H, s), 6.81 (1H, t, $^3J_{HF} = 9$ Hz) ppm; ^{13}C -NMR (100 MHz, DMSO- d_6): δ = 163.0 (dd, $^1J_{CF} = 243$ Hz, $^3J_{CF} = 17$ Hz, C), 152.0 (C), 150.6 (C), 142.5 (t, $^3J_{CF} = 14$ Hz, C), 138.7 (C), 138.4 (C), 133.4 (C), 129.8 (CH), 129.1 (CH), 128.4 (CH), 128.3 (CH), 125.6 (CH), 124.9 (CH), 101.5 (dd, $^2J_{CF} = 21$ Hz, $^4J_{CF} = 8$ Hz, CH), 97.6 (2CH) ppm; MS (EI pos.): m/z (%) = 390.0 (47) ($[M]^+$, calcd. 390.1), 235.2 (100) ($[M_{fr.}]^+$, calcd. 235.2); HRMS (ESI pos.): m/z = 391.1365 $[M+H]^+$, calcd. for $[C_{22}H_{17}F_2N_4O]^+ = 391.1365$.

5.1.53 1-(3-(*tert*-Butyl)-1-(4-nitrophenyl)-1*H*-pyrazol-5-yl)-3-(4-chlorophenyl)urea (**49**).

Prepared according to general procedure D, 3-(*tert*-butyl)-1-(4-nitrophenyl)-1*H*-pyrazol-5-amine (**10**, 2.00 g, 7.68 mmol) and 1-chloro-4-isocyanatobenzene (1.07 g, 6.99 mmol) were dissolved in CH_2Cl_2 (50 mL) and the mixture was stirred at rt for 20 h. The resultant solid was filtered and washed with hexane (30 mL). Subsequently, the filtrate was concentrated under reduced pressure to enforce crystallizing of additional product, which was then filtered and washed with hexane to yield 1.83 g (4.41 mmol, 81%) of **49** as a yellow solid. TLC: $R_F = 0.89$ (SiO₂, hexane/EtOAc 1:3); HPLC: $t_R = 16.83$ min; 1H -NMR (500 MHz, DMSO- d_6): δ = 9.20 (s, 1H), 8.67 (s, 1H), 8.36 (d, $^3J = 9.2$ Hz, 2H), 7.88 (d, $^3J = 9.2$ Hz, 2H), 7.44 (d, $^3J = 8.9$ Hz, 2H), 7.30 (d, $^3J = 8.9$ Hz, 2H), 6.45 (s, 1H), 1.30 (s, 9H) ppm; ^{13}C -NMR (126 MHz, DMSO- d_6): δ = 162.5, 151.9, 145.0, 144.0, 138.3, 137.8, 128.7, 125.8, 124.9, 123.2, 119.9, 99.9, 32.2,

29.9 ppm; MS (ESI pos.): m/z (%) = 414.2 (100) ($[M+H]^+$, calcd. 414.2); HRMS (FTMS +p MALDI): m/z = 414.1345 $[M+H]^+$, calcd. for $[C_{20}H_{21}ClN_5O_3]^+$ = 414.1327.

5.1.54 1-(3-(*tert*-Butyl)-1-(4-(trifluoromethyl)phenyl)-1*H*-pyrazol-5-yl)-3-(4-chlorophenyl)urea (50).

Prepared according to general procedure D, 3-(*tert*-butyl)-1-(4-(trifluoromethyl)phenyl)-1*H*-pyrazol-5-amine (**11**, 460 mg, 1.62 mmol) and 1-chloro-4-isocyanatobenzene (340 mg, 2.21 mmol) in CH_2Cl_2 (10 mL) were stirred at rt for 20 h. The resultant solid was filtered and washed with hexane (15 mL). Subsequently, the filtrate was concentrated under reduced pressure to crystallize additional product, which was filtered and washed with hexane. The combined crude product was recrystallized from hexane/EtOAc (1:1) to yield 226 mg (0.52 mmol, 32%) of **50** as a colorless solid. TLC: R_F = 0.91 (SiO_2 , cyclohexane/EtOAc 1:3); HPLC: t_R = 17.5 min; 1H -NMR (500 MHz, DMSO- d_6): δ = 9.16 (br s, 1H), 8.59 (br s, 1H), 7.89 (d, 3J = 8.3 Hz, 2H), 7.81 (d, 3J = 8.3 Hz, 2H), 7.45 (d, 3J = 8.5 Hz, 2H), 7.31 (d, 3J = 8.3 Hz, 2H), 6.43 (br s, 1H), 1.29 (s, 9H); ^{13}C -NMR (126 MHz, DMSO- d_6): δ = 161.8, 151.8, 142.0, 138.4, 137.5, 128.7, 127.0 ($^2J_{CF}$ = 32.2 Hz), 126.4, 125.8, 124.6 ($^1J_{CF}$ = 274.9 Hz), 123.9, 119.9, 97.5, 30.0 ppm; MS (ESI pos.): m/z (%) = 437.2 (100) ($[M+H]^+$, calcd. 437.1); 310.2 (31) ($[M_{fr.I}]^+$, calcd. 310.1); 284.2 (14) ($[M_{fr.II}+H]^+$, calcd. 284.1); HRMS (FTMS +p MALDI): m/z = 437.1330 $[M+H]^+$, calcd. for $[C_{21}H_{21}ClF_3N_4O]^+$ = 437.1351.

5.1.55 1-(3-(*tert*-Butyl)-1-(4-(trifluoromethoxy)phenyl)-1*H*-pyrazol-5-yl)-3-(4-chlorophenyl)urea (51).

Prepared according to general procedure D, 3-(*tert*-butyl)-1-(4-(trifluoromethoxy)phenyl)-1*H*-pyrazol-5-amine (**12**, 505 mg, 1.69 mmol) and 1-chloro-4-isocyanatobenzene (236 mg, 1.53 mmol) in CH_2Cl_2 (10 mL) were stirred at rt for 20 h. The resultant solid was filtered and washed with hexane (15 mL). Subsequently, the filtrate was concentrated under reduced pressure to crystallize additional product, which was filtered and washed with hexane. The combined crude product was recrystallized from hexane/EtOAc

(1:1) to yield 480 mg (1.06 mmol, 69%) of **51** as a colorless solid; TLC: R_F = 0.92 (SiO₂, cyclohexane/EtOAc 1:3); HPLC: t_R = 17.6 min; ¹H-NMR (600 MHz, DMSO-*d*₆): δ = 9.12 (br s, 1H), 8.51 (br s, 1H), 7.67 (d, ³*J* = 8.9 Hz, 2H), 7.52 (d, ³*J* = 8.9 Hz, 2H), 7.44 (d, ³*J* = 8.8 Hz, 2H), 7.31 (d, ³*J* = 8.8 Hz, 2H), 6.39 (s, 1H), 1.28 (s, 9H) ppm; ¹³C-NMR (150 MHz, DMSO-*d*₆): δ = 161.3, 151.7, 146.8, 138.3, 137.7, 137.2, 128.6, 125.7 (2x), 121.9, 120.1 (¹*J*_{CF} = 259 Hz), 119.8, 96.5, 32.0, 30.1 ppm; MS (ESI pos.): *m/z* (%) = 453.2 (100) ([M+H]⁺, calcd. 453.1); 326.2 (37) ([M_{fr.I}]⁺, calcd. 326.1); 300.2 (11) ([M+H]⁺, calcd. 300.1); HRMS (FTMS +p MALDI): *m/z* = 453.1308 [M+H]⁺, calcd. for [C₂₁H₂₁ClF₃N₄O₂]⁺ = 453.1300.

5.1.56 1-(3-(*tert*-Butyl)-1-(4-cyanophenyl)-1*H*-pyrazol-5-yl)-3-(4-chlorophenyl)urea (**52**).

Prepared according to general procedure D, 4-(5-amino-3-(*tert*-butyl)-1*H*-pyrazol-1-yl)benzonitrile (**13**, 590 mg, 2.46 mmol) and 1-chloro-4-isocyanatobenzene (514 mg, 3.35 mmol) in CH₂Cl₂ (30 mL) were stirred at rt for 20 h. The resultant solid was filtered and washed with hexane (15 mL). Subsequently, the filtrate was concentrated under reduced pressure to crystallize additional product, which was filtered and washed with hexane. The combined crude product was recrystallized from hexane/EtOAc (1:1) to yield 539 mg (1.37 mmol, 56%) of **52** as a yellow solid. TLC: R_F = 0.89 (SiO₂, cyclohexane/EtOAc 1:3); HPLC: t_R = 16.5 min; ¹H-NMR (600 MHz, DMSO-*d*₆): δ = 9.18 (br s, 1H), 8.60 (br s, 1H), 7.98 (d, ³*J* = 8.6 Hz, 2H), 7.80 (d, ³*J* = 8.6 Hz, 2H), 7.44 (d, ³*J* = 8.8 Hz, 2H), 7.31 (d, ³*J* = 8.8 Hz, 2H), 6.42 (s, 1H), 1.29 (s, 9H) ppm; ¹³C-NMR (150 MHz, DMSO-*d*₆): δ = 162.1, 151.9, 142.4, 138.3, 137.5, 133.5, 128.8, 125.6, 123.4, 119.8, 118.5, 108.8, 98.5, 32.1, 29.9 ppm; MS (ESI pos.): *m/z* (%) = 394.2 (100) ([M+H]⁺, calcd. 394.2); 267.2 (32) ([M_{fr.I}-H]⁺, calcd. 268.1); 241.2 (29) ([M_{fr.II}+H]⁺, calcd. 241.2); HRMS (FTMS +p MALDI): *m/z* = 394.1409 [M+H]⁺, calcd. for [C₂₁H₂₁ClN₅O]⁺ = 394.1429.

5.1.57 1-(3-(*tert*-Butyl)-1-(4-(methylsulfonyl)phenyl)-1*H*-pyrazol-5-yl)-3-(4-chlorophenyl)urea (**53**).

Prepared according to general procedure D, 3-(*tert*-butyl)-1-(4-(methylsulfonyl)phenyl)-1*H*-pyrazol-5-amine (**14**, 700 mg, 2.39 mmol) and 1-chloro-4-isocyanatobenzene (333 mg, 2.17 mmol) in CH₂Cl₂

(15 mL) were stirred at rt for 20 h. The resultant solid was filtered and washed with hexane (15 mL). Subsequently, the filtrate was concentrated under reduced pressure to crystallize additional product, which was filtered and washed with hexane. The combined crude product was recrystallized from hexane/EtOAc (1:1) to yield 761 mg (1.70 mmol, 78%) of **53** as a yellowish solid. TLC: R_F = 0.72 (SiO₂, cyclohexane/EtOAc 1:3); HPLC: t_R = 15.8 min; ¹H-NMR (600 MHz, DMSO-*d*₆): δ = 9.17 (br s, 1H), 8.63 (br s, 1H), 8.06 (d, ³*J* = 8.7 Hz, 2H), 7.84 (d, ³*J* = 8.7 Hz, 2H), 7.45 (d, ³*J* = 8.9 Hz, 2H), 7.31 (d, ³*J* = 8.9 Hz, 2H), 6.43 (s, 1H), 3.27 (s, 3H), 1.29 (s, 9H) ppm; ¹³C-NMR (150 MHz, DMSO-*d*₆): δ = 162.0, 151.8, 142.6, 138.5, 138.3, **137.5**, 128.6, 128.3, 125.7, 123.6, 119.8, 97.8, 43.5, 32.1, 30.0 ppm; MS (ESI pos.): *m/z* (%) = 447.2 (100) ([*M*+H]⁺, calcd. 447.1); 294.2 (28) ([*M*_{fr.I}+H]⁺, calcd. 294.1); 320.2 (29) ([*M*_{fr.II}]⁺, calcd. 320.1); HRMS (FTMS +p MALDI): *m/z* = 469.1054 [*M*+Na]⁺, calcd. for [C₂₁H₂₃ClN₄NaO₃S]⁺ = 469.1072.

5.1.58 1-(4-Chlorophenyl)-3-(3-cyclopropyl-1-(4-(trifluoromethoxy)phenyl)-1*H*-pyrazol-5-yl)urea (**54**).

Prepared according to general procedure D using 3-cyclopropyl-1-(4-(trifluoromethoxy)phenyl)-1*H*-pyrazol-5-amine (**15**, 500 mg, 1.77 mmol) and 1-chloro-4-isocyanatobenzene (407 mg, 2.65 mmol) in CH₂Cl₂ (20 mL) which were stirred at rt for 20 h. The crude product was recrystallized from hexane/EtOAc (1:1) to yield 605 mg (1.39 mmol, 78%) of **54** as a colorless solid. TLC: R_F = 0.94 (SiO₂, cyclohexane/EtOAc 1:3); HPLC: t_R = 16.4 min; ¹H-NMR (500 MHz, DMSO-*d*₆): δ = 9.09 (br s, 1H), 8.53 (br s, 1H), 7.65 (d, ³*J* = 9.0 Hz, 2H), 7.51 (d, ³*J* = 9.0 Hz, 2H), 7.43 (d, ³*J* = 8.9 Hz, 2H), 7.30 (d, ³*J* = 8.9 Hz, 2H), 6.19 (s, 1H), 1.93–1.86 (m, 1H), 0.92–0.87 (m, 2H), 0.73–0.68 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆): δ = 154.8, 151.7, 146.8, 138.3, 137.5 (2x), 128.6, 125.7, 122.0, 120.1 (¹*J*_{CF} = 256.9 Hz), 119.8, 96.4, 9.4, 7.8 ppm; MS (ESI pos.): *m/z* (%) = 436.9 (100) ([*M*+H]⁺, calcd. 437.1); HRMS (FTMS +p MALDI): *m/z* = 437.0978 [*M*+H]⁺, calcd. for [C₂₀H₁₇ClF₃N₄O₂]⁺ = 437.0987.

5.1.59 1-(4-Chlorophenyl)-3-(1-(4-cyanophenyl)-3-cyclopropyl-1*H*-pyrazol-5-yl)urea (**55**).

Prepared according to general procedure D using 4-(5-amino-3-cyclopropyl-1*H*-pyrazol-1-yl)benzonitrile (**16**, 500 mg, 2.23 mmol) and 1-chloro-4-isocyanatobenzene (514 mg, 3.35 mmol), in CH₂Cl₂ (20 mL) which were stirred at rt for 20 h. The crude product was recrystallized from hexane/EtOAc (1:1) to yield 110 mg (0.29 mmol, 13%) of **55** as a colorless solid. TLC: R_F = 0.86 (SiO₂, cyclohexane/EtOAc 1:3); HPLC: t_R = 15.3 min; ¹H-NMR (600 MHz, DMSO-*d*₆): δ = 9.14 (s, 1H), 8.62 (s, 1H), 7.97 (d, ³*J* = 8.4 Hz, 2H), 7.77 (d, ³*J* = 8.4 Hz, 2H), 7.43 (d, ³*J* = 8.6 Hz, 2H), 7.30 (d, ³*J* = 8.6 Hz, 2H), 6.21 (s, 1H), 1.96–1.87 (m, 1H), 0.96–0.88 (m, 2H), 0.77–0.68 (m, 2H) ppm; ¹³C-NMR (150 MHz, DMSO-*d*₆): δ = 155.7, 151.9, 142.4, 138.3, 137.7, 133.4, 128.8, 125.6, 123.4, 119.9, 118.4, 108.8, 98.3, 9.3, 7.8 ppm; MS (ESI pos.): m/z (%) = 377.9 (100) ([*M*+*H*]⁺, calcd. 378.1); 225.1 (63) ([*M*_{fr.}+2*H*]⁺, calcd. 225.0); HRMS (FTMS +p MALDI): m/z = 378.1100 [*M*+*H*]⁺, calcd. for [C₂₀H₁₇ClN₅O]⁺ = 378.1116.

5.1.60 1-(4-Chlorophenyl)-3-(3-cyclopropyl-1-(4-nitrophenyl)-1*H*-pyrazol-5-yl)urea (**56**).

Prepared according to general procedure D using 3-cyclopropyl-1-(4-nitrophenyl)-1*H*-pyrazol-5-amine (**17**, 500 mg, 2.05 mmol) and 1-chloro-4-isocyanatobenzene (314 mg, 2.05 mmol) in CH₂Cl₂ (20 mL) which were stirred at rt for 20 h. The crude product was recrystallized from hexane/EtOAc (1:1) to yield 262 mg (0.66 mmol, 32%) of **56** as a colorless solid. TLC: R_F = 0.88 (SiO₂, cyclohexane/EtOAc 98:2); HPLC: t_R = 15.65 min; ¹H-NMR (500 MHz, DMSO-*d*₆): δ = 9.16 (s, 1H), 8.69 (s, 1H), 8.35 (d, ³*J* = 8.9 Hz, 2H), 7.87 (d, ³*J* = 8.9 Hz, 2H), 7.43 (d, ³*J* = 8.8 Hz, 2H), 7.30 (d, ³*J* = 8.8 Hz, 2H), 5.75 (s, 1H), 1.98–1.88 (m, 1H), 0.98–0.86 (m, 2H), 0.77–0.68 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆): δ = 156.2, 151.9, 145.0, 143.8, 138.3, 138.0, 128.6, 125.8, 124.8, 123.1, 119.9, 98.8, 39.5, 9.4, 7.9 ppm; MS (ESI pos.): m/z (%) = 397.94 (100) ([*M*+*H*]⁺, calcd. 398.10); HRMS (FTMS +p MALDI): m/z = 398.1015 [*M*+*H*]⁺, calcd. for [C₁₉H₁₇ClN₅O₃]⁺ = 398.1014.

5.1.61 1-(4-Chlorophenyl)-3-(1-(4-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-5-yl)urea (**57**).

Prepared according to general procedure D using 1-(4-nitrophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-5-amine (**61**, 1.00 g, 3.67 mmol) and 1-chloro-4-isocyanatobenzene (769 mg, 5.01 mmol) in CH₂Cl₂

(35 mL) which were stirred at rt for 20 h. The crude product was recrystallized from cyclohexane/EtOAc (1:1) to yield 122 mg (0.29 mmol, 8%) of **57** as a brownish solid. TLC: R_F = 0.91 (SiO₂, cyclohexane/EtOAc 1:3); HPLC: t_R = 16.32 min; ¹H-NMR (500 MHz, DMSO-d₆): δ = 9.26 (s, 1H), 8.98 (s, 1H), 8.44 (d, ³ J = 8.9 Hz, 2H), 7.95 (d, ³ J = 8.9 Hz, 2H), 7.43 (d, ³ J = 8.7 Hz, 2H), 7.32 (d, ³ J = 8.7 Hz, 2H), 6.95 (s, 1H) ppm; ¹³C-NMR (126 MHz, DMSO-d₆): δ = 149.7, 145.6, 143.4, 142.6 (² J_{CF} = 36.9 Hz), 126.4, 124.9, 123.7, 121.4 (¹ J_{CF} = 269.2 Hz), 112.4, 87.8 ppm; MS (ESI pos.): m/z (%) = 425.8 (17) ([M+H]⁺, calcd. 426.1); 357.1 (12) ([M_{fr,II}]⁺, calcd. 357.1); 256.9 (33) ([M_{fr,I}]⁺, calcd. 256.0); HRMS (FTMS +p MALDI): m/z = 426.0570 [M+H]⁺, calcd. for [C₁₇H₁₂ClF₃N₅O₃]⁺ = 426.0575.

5.1.62 1-(4-Nitrophenyl)-3-(trifluoromethyl)-1H-pyrazol-5-amine (**61**).

Potassium *tert*-butoxide (3.25 g, 28.96 mmol) was dissolved in THF (dry, 30 mL) and acetonitrile (0.39 mg, 9.60 mmol). Ethyl 2,2,2-trifluoroacetate (**58**, 5.44 g, 38.29 mmol) was added dropwise and the mixture was stirred overnight at rt. The mixture was then acidified with hydrochloric acid (conc., 3 mL) and water (10 mL) was added. The solvent was removed under reduced pressure and the residue was extracted with EtOAc (3 x 20 mL). The organic extracts were combined, dried over MgSO₄, and the solvent was removed under reduced pressure to give 4,4,4-trifluoro-3-oxobutanenitrile (**59**), which was used without further purification. The crude compound was dissolved in EtOH (abs., 20 mL), and (4-nitrophenyl)hydrazine hydrochloride (**60**, 1.81 g, 9.57 mmol) was added. The mixture was heated for 8 h at 70 °C and afterwards basified to pH~12 with aqueous NaOH solution (3N, 10 mL). The mixture was extracted with EtOAc (4 x 50 mL), and the organic extracts were combined and dried over MgSO₄. After removing the solvent under reduced pressure, the crude product was purified by column chromatography on silica (cyclohexane/EtOAc 4:1 → 1:1 → 1:20 → EtOAc) to yield 1.19 g (4.37 mmol, 46%) of **61** as an orange solid. TLC: R_F = 0.63 (SiO₂, cyclohexane/EtOAc 1:1); ¹H-NMR (500 MHz, DMSO-d₆): δ = 8.38 (d, ³ J = 9.1 Hz, 2H), 7.94 (d, ³ J = 9.1 Hz, 2H), 6.10 (s, 2H), 5.88 (s, 1H) ppm; ¹³C-NMR (126 MHz, DMSO-d₆): δ = 149.7, 145.6, 143.4, 142.6 (² J_{CF} = 36.9 Hz), 126.4, 124.9, 123.7, 121.4 (¹ J_{CF} = 269.2 Hz), 112.4, 87.8 ppm; MS (ESI pos.): m/z (%) = 273.0 (100) ([M+H]⁺, calcd. 273.1).

5.1.63 1-(1-(4-Aminophenyl)-3-(*tert*-butyl)-1*H*-pyrazol-5-yl)-3-(4-chlorophenyl)urea (**62**).

1-(3-(*tert*-Butyl)-1-(4-nitrophenyl)-1*H*-pyrazol-5-yl)-3-(4-chlorophenyl)urea (**49**, 1.14 g, 2.76 mmol) was dissolved in an EtOH/H₂O mixture (4:1, 3 mL). Subsequently, Fe powder (1.54 g, 27.55 mmol) was sprinkled on, NH₄Cl (1.47 g, 27.55 mmol) was added and the reaction was heated for 1 h at 70 °C. The mixture was filtered over Celite[®] and washed with EtOAc. The crude product was purified by column chromatography on silica (cyclohexane/EtOAc: 1:1 → 1:3) to yield 782 mg (2.03 mmol, 74%) of **62** as a colorless solid. TLC: *R*_F = 0.42 (SiO₂, cyclohexane/EtOAc 1:3); HPLC: *t*_R = 15.23 min; ¹H-NMR (500 MHz, DMSO-*d*₆): δ = 9.19 (s, 1H), 8.20 (s, 1H), 7.42 (d, ³*J* = 8.9 Hz, 2H), 7.30 (d, ³*J* = 8.9 Hz, 2H), 7.07 (d, ³*J* = 8.7 Hz, 2H), 6.66 (d, ³*J* = 8.7 Hz, 2H), 6.30 (s, 1H), 5.40 (br s, 2H), 1.25 (s, 9H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆): δ = 159.6, 151.1, 148.7, 138.5, 137.1, 128.7, 126.6, 126.5, 125.5, 119.5, 113.7, 92.8, 32.0, 30.3 ppm; MS (ESI pos.): *m/z* (%) = 384.2 (100) ([*M*+H]⁺, calcd. 384.2); 257.2 (49) ([*M*_{fr.I}]⁺, calcd. 257.3); 231.1 (24) ([*M*_{fr.II}+H]⁺, calcd. 231.2); HRMS (FTMS +p MALDI): *m/z* = 384.1570 [*M*+H]⁺, calcd. for [C₂₀H₂₃ClN₅O₃]⁺ = 384.1586.

5.1.64 *N*-(4-(3-(*tert*-Butyl)-5-(3-(4-chlorophenyl)ureido)-1*H*-pyrazol-1-yl)phenyl)cyclopropanecarboxamide (**63**).

Cyclopropanecarboxylic acid (28 mg, 0.33 mmol) and HATU (149 mg, 0.39 mmol) were dissolved in DMF (dry, 8 mL) and DIPEA (51 mg, 0.39 mmol) was added. The mixture was stirred at rt for 1.5 h and a solution of 1-(1-(4-aminophenyl)-3-(*tert*-butyl)-1*H*-pyrazol-5-yl)-3-(4-chlorophenyl)urea (**62**, 150 mg, 0.39 mmol) in DMF (dry, 2 mL) was added. The mixture was then stirred for additional 20 h at rt. Water (20 mL) was added, the precipitated solid was filtered, washed with water (200 mL) and dried in a vacuum oven to yield 89 mg (0.20 mmol, 50%) of **63** as a colorless solid. TLC: *R*_F = 0.64 (SiO₂, cyclohexane/EtOAc 1:3); HPLC: *t*_R = 15.92 min; ¹H-NMR (300 MHz, DMSO-*d*₆): δ = 10.37 (br s, 1H), 9.13 (br s, 1H), 8.35 (br s, 1H), 7.73 (d, ³*J* = 8.9 Hz, 2H), 7.40–7.38 (m, 4H), 7.30 (d, ³*J* = 8.9 Hz, 2H), 6.35 (s, 1H), 1.86–1.73 (m, 1H), 1.27 (s, 9H), 0.90–0.74 (m, 4H) ppm; ¹³C-NMR (75 MHz, DMSO-*d*₆): δ = 171.8, 160.5, 151.4, 138.6, 138.4, 137.0, 133.2, 128.6, 125.6, 125.1, 119.6, 119.4, 94.8, 32.0, 30.2, 14.6, 7.3

ppm; MS (ESI pos.): m/z (%) = 452.18 (100) ($[M+H]^+$, calcd. 452.19); MS (ESI neg.): m/z (%) = 450.26 (100) ($[M-H]^-$, calcd. 450.17); HRMS (FTMS +p MALDI): m/z = 452.1836 $[M+H]^+$, calcd. for $[C_{24}H_{27}ClN_5O_2]^+$ = 452.1848.

5.1.65 *N*-(4-(3-(*tert*-Butyl)-5-(3-(4-chlorophenyl)ureido)-1*H*-pyrazol-1-yl)phenyl)isobutyramide (64).

1-(1-(4-Aminophenyl)-3-(*tert*-butyl)-1*H*-pyrazol-5-yl)-3-(4-chlorophenyl)urea (**62**, 300 mg, 0.78 mmol) was dissolved in THF (dry, 10 mL) and TEA (158 mg, 1.56 mmol) was added. The mixture was cooled to 0 °C and isobutyryl chloride (166 mg, 1.56 mmol) was added dropwise. After warming to rt, the mixture was stirred for 20 h at rt. Subsequently, the solvent was removed under reduced pressure and the residue was dissolved in EtOAc. The crude product was purified by column chromatography on silica (cyclohexane/EtOAc: 1:10 → 1:1 → 1:3 → 1:20 → EtOAc) to yield 298 mg (0.66 mmol, 84%) of **64** as a colorless solid. TLC: R_F = 0.75 (SiO₂, cyclohexane/EtOAc 1:3); HPLC: t_R = 16.12 min; ¹H-NMR (500 MHz, DMSO-*d*₆): δ = 10.03 (br s, 1H), 9.14 (br s, 1H), 8.33 (br s, 1H), 7.75 (d, ³*J* = 8.9 Hz, 2H), 7.45–7.39 (m, 4H), 7.30 (d, ³*J* = 8.9 Hz, 2H), 6.35 (s, 1H), 2.68–2.57 (m, 1H), 1.27 (s, 9H), 1.11 (d, ³*J* = 6.7 Hz, 6H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆): δ = 175.4, 160.5, 151.4, 138.8, 138.4, 137.1, 133.2, 128.7, 125.6, 125.1, 119.6, 119.5, 94.7, 35.0, 32.0, 30.72, 19.5 ppm; MS (ESI pos.): m/z (%) = 454.2 (100) ($[M+H]^+$, calcd. 454.2); 327.2 (28) ($[M_{fr.1}]^+$, calcd. 327.2); HRMS (FTMS +p MALDI): m/z = 545.2008 $[M+H]^+$, calcd. for $[C_{24}H_{29}ClN_5O_2]^+$ = 454.2004.

5.1.66 *N*-(4-(3-(*tert*-Butyl)-5-(3-(4-chlorophenyl)ureido)-1*H*-pyrazol-1-yl)phenyl)methanesulfonamide (65).

1-(1-(4-Aminophenyl)-3-(*tert*-butyl)-1*H*-pyrazol-5-yl)-3-(4-chlorophenyl)urea (**62**, 200 mg, 0.52 mmol) was dissolved in THF (dry, 8 mL) and TEA (53 mg, 0.52 mmol) was added. The mixture was cooled to 0 °C, and methanesulfonyl chloride (179 mg, 1.56 mmol) was added dropwise. After warming to rt, the mixture was stirred for 20 h at rt. Subsequently, the solvent was removed under reduced pressure, and the residue was dissolved in EtOAc. The organic phase was washed 4-times with water and dried over

MgSO₄. The crude product was purified by column chromatography on silica (cyclohexane/EtOAc: 1:10 → 1:20) to yield 70 mg (0.15 mmol, 29%) of **65** as a colorless solid. TLC: R_F = 0.70 (SiO₂, cyclohexane/EtOAc 1:3); HPLC: t_R = 15.44 min; ¹H-NMR (400 MHz, DMSO-d₆, 300 K): δ = 9.98 (br s, 1H), 9.12 (br s, 1H), 8.40 (br s, 1H), 7.52–7.40 (m, 4H) 7.38–7.30 (m, 4H), 6.36 (s, 1H), 3.05 (s, 3H), 1.27 (s, 9H) ppm; ¹³C-NMR (101 MHz, DMSO-d₆, 300 K): δ = 160.6, 151.4, 138.4, 137.6, 137.1, 134.2, 128.7, 125.7, 125.6, 120.0, 119.7, 95.0, 32.0, 30.2 ppm; MS (ESI pos.): m/z (%) = 461.97 (100) ([M+H]⁺, calcd. 462.12); HRMS (FTMS +p MALDI): m/z = 462.1348 [M+H]⁺, calcd. for [C₂₁H₂₅ClN₅O₃S]⁺ = 462.1361.

5.1.67 Ethyl 5-amino-1-phenyl-1*H*-pyrazole-4-carboxylate (**67**).

Prepared as previously published by Röhm et al. [6].

5.1.68 5-Amino-1-phenyl-1*H*-pyrazole-4-carboxylic acid (**68**).

Prepared as previously published by Röhm et al. [6].

5.1.69 Methyl 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoate (**69**).

Prepared as previously published by Röhm et al. [6] 5-Amino-1-phenyl-1*H*-pyrazole-4-carboxylic acid (**68**, 2.00 g, 9.84 mmol), HOBt (1.56 g, 11.81 mmol) and EDC-HCl (2.26 g, 11.81 mmol) were dissolved in DMF (dry, 20 mL) and DIPEA (1.91 g, 14.77 mmol) was added. The mixture was stirred for 30 min at rt and a solution of methyl 4-(aminomethyl)benzoate hydrochloride (1.95 g, 11.81 mmol) in DMF/DMSO (dry, 20 mL, 1:1) and DIPEA (1.91 g, 14.77 mmol) was added. The reaction mixture was stirred for 20 h at rt., CH₂Cl₂ (100 mL) was added, and the mixture was washed with H₂O (5x50 mL). The organic phase was dried over MgSO₄, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica (CH₂Cl₂/MeOH, 98:2) to afford 3.10 g (8.85 mmol, 90%) of **69** as a colorless solid. TLC: R_F = 0.33 (SiO₂, CH₂Cl₂/MeOH 10:1); ¹H-NMR (400 MHz, DMSO-d₆, 300 K): δ = 8.54 (t, ³ J = 6.1 Hz, 1H), 7.99 (s, 1H), 7.94 (d, ³ J = 8.2 Hz, 2H), 7.60–7.49 (m, 4H), 7.45 (d, ³ J = 8.2 Hz, 2H), 7.42–7.36 (m, 1H), 6.38 (s, 2H), 4.50 (d, ³ J = 6.1 Hz, 2H), 3.85 (s, 3H) ppm; ¹³C-NMR (101 MHz, DMSO-d₆, 300 K): δ = 166.1, 164.2, 149.2, 145.9, 138.4, 138.2, 129.3, 129.2, 128.0, 127.3, 127.1, 123.1, 97.3, 52.0, 41.3 ppm; MS (ESI pos.): m/z (%) = 351.13 (100) ([M+H]⁺, calcd. 351.15).

5.1.70 4-((5-Amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**).

Prepared as previously published by Röhme et al. [6].

5.1.71 (*S*)-(9*H*-Fluoren-9-yl)methyl (4-cyclohexyl-1-(methylamino)-1-oxobutan-2-yl)carbamate (**72**).

Compound **72** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 400 mg, 0.98 mmol) and methanamine (596 μ L, 2M in THF, 1.18 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc, 1:1) to afford 334 mg (0.80 mmol, 81%) of **72** as a colorless solid. TLC: R_F = 0.40 (SiO₂, cyclohexane/EtOAc, 1:1); ¹H-NMR (500 MHz, CDCl₃, 300 K): δ = 7.76 (d, ³*J* = 7.5 Hz, 2H), 7.62–7.54 (m, 2H), 7.40 (t, ³*J* = 7.5 Hz, 2H), 7.30 (t, ³*J* = 7.5 Hz, 2H), 6.11 (s, 1H), 5.45 (d, ³*J* = 8.0 Hz, 1H), 4.46–4.33 (m, 2H), 4.26–4.17 (m, 1H), 4.11–4.01 (m, 1H), 2.81 (s, 3H), 1.89–1.79 (m, 1H), 1.72–1.56 (m, 6H), 1.30–1.05 (m, 6H, H-1), 0.93–0.78 (m, 2H) ppm; ¹³C-NMR (126 MHz, CDCl₃, 300 K): δ = 172.6, 156.4, 143.9, 143.8, 141.4, 127.9, 127.2 (2x), 125.2, 120.1, 67.1, 55.5, 47.3, 38.8, 37.6, 33.4, 33.3, 33.2, 30.2, 26.7, 26.4 (2x) ppm; MS (ESI pos.): *m/z* (%) = 421.35 (100) ([M+H]⁺, calcd. 421.24), 199.24 (66) ([M-Fmoc+H]⁺, calcd. 199.17), 179.21 (29) ([M_{fr.}+H]⁺, calcd. 179.08).

5.1.72 (*S*)-(9*H*-Fluoren-9-yl)methyl (4-cyclohexyl-1-(ethylamino)-1-oxobutan-2-yl)carbamate (**73**).

Compound **73** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 400 mg, 0.98 mmol) and ethanamine (596 μ L, 2M in THF, 1.18 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc, 1:1) to afford 224 mg (0.52 mmol, 52%) of **73** as a colorless solid. TLC: R_F = 0.68 (SiO₂, cyclohexane/EtOAc, 1:1); ¹H-NMR (500 MHz, CDCl₃, 300 K): δ = 7.76 (d, ³*J* = 7.5 Hz, 2H), 7.58 (d, ³*J* = 7.5 Hz, 2H), 7.40 (t, ³*J* = 7.5 Hz, 2H), 7.31 (t, ³*J* = 7.5 Hz, 2H), 5.89 (s, 1H), 5.36 (d, ³*J* = 7.7 Hz, 1H), 4.46–4.35 (m, 2H), 4.22 (t, ³*J* = 6.9 Hz, 1H), 4.08–3.99 (m, 1H), 3.36–3.22 (m, 2H), 1.90–1.78 (m, 1H), 1.74–1.56 (m, 6H), 1.34–1.05 (m, 9H), 0.94–0.79 (m, 2H) ppm; ¹³C-NMR (126 MHz, CDCl₃, 300 K): δ = 171.6, 154.3, 143.9, 141.4, 127.9, 127.2, 125.2, 120.1, 67.1, 55.5, 47.3, 37.6, 37.5, 34.6, 33.4, 33.3,

33.2, 30.2, 26.7, 26.4, 14.9 ppm; MS (ESI pos.): m/z (%) = 435.26 (100) ($[M+H]^+$, calcd. 435.27), 213.29 (52) ($[M-Fmoc+H]^+$, calcd. 213.20), 179.29 (28) ($[M_{fr.}+H]^+$, calcd. 179.29).

5.1.73 (*S*)-(9*H*-Fluoren-9-yl)methyl (4-cyclohexyl-1-oxo-1-(propylamino)butan-2-yl)carbamate (**74**).

Compound **74** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 400 mg, 0.98 mmol) and propan-1-amine (97 μ L, 1.18 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc, 1:1) to afford 248 mg (0.55 mmol, 56%) of **74**. TLC: R_F = 0.15 (SiO₂, cyclohexane/EtOAc, 1:1); ¹H-NMR (500 MHz, CDCl₃, 300 K): δ = 7.76 (d, ³ J = 7.5 Hz, 2H), 7.57 (d, ³ J = 7.5 Hz, 2H), 7.39 (t, ³ J = 7.4 Hz, 2H), 7.30 (t, ³ J = 7.4 Hz, 2H), 6.13 (s, 1H), 5.48 (d, ³ J = 7.9 Hz, 1H), 4.48–4.28 (m, 2H), 4.20 (t, ³ J = 7.0 Hz, 1H), 4.12–4.02 (m, 1H), 3.30–3.11 (m, 2H), 1.91–1.76 (m, 1H), 1.73–1.58 (m, 6H), 1.56–1.43 (m, 2H), 1.36–1.06 (m, 6H), 0.94–0.79 (m, 5H) ppm; ¹³C-NMR (126 MHz, CDCl₃, 300 K): δ = 171.8, 156.3, 143.9, 141.4, 127.9, 127.2, 125.2, 120.1, 67.1, 55.5, 47.2, 41.3, 37.6, 33.4, 33.3, 33.1, 30.4, 26.7, 26.4, 22.9, 11.4 ppm; MS (ESI pos.): m/z (%) = 897.62 (84) ($[2M+H]^+$, calcd. 897.54), 449.24 (92) ($[M+H]^+$, calcd. 449.27), 227.18 (100) ($[M-Fmoc+H]^+$, calcd. 227.20).

5.1.74 (*S*)-(9*H*-Fluoren-9-yl)methyl (1-(butylamino)-4-cyclohexyl-1-oxobutan-2-yl)carbamate (**75**).

Compound **75** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 400 mg, 0.98 mmol) and butan-1-amine (120 μ L, 1.18 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc, 4:1 $\rightarrow$ 1:1) to afford 301 mg (0.65 mmol, 66%) of **75** as a colorless solid. TLC: R_F = 0.78 (SiO₂, cyclohexane/EtOAc, 1:1); ¹H-NMR (500 MHz, CDCl₃, 300 K): δ = 7.76 (d, ³ J = 7.5 Hz, 2H), 7.57 (d, ³ J = 7.5 Hz, 2H), 7.40 (t, ³ J = 7.3 Hz, 2H), 7.30 (t, ³ J = 7.3 Hz, 2H), 6.03 (s, 1H), 5.48–5.37 (m, 1H), 4.45–4.32 (m, 2H), 4.21 (t, ³ J = 7.0 Hz, 1H), 4.11–4.00 (m, 1H), 3.33–3.14 (m, 2H), 1.91–1.78 (m, 1H), 1.74–1.56 (m, 6H), 1.52–1.39 (m, 2H), 1.37–1.05 (m, 8H), 0.93–0.79 (m, 5H) ppm; ¹³C-NMR (126 MHz, CDCl₃, 300 K): δ = 171.7, 156.3, 143.9, 141.4, 127.9, 127.2, 125.2, 120.1, 67.1, 55.5, 47.3, 39.4, 37.6, 33.4, 33.3,

33.1, 31.7, 30.4, 26.7, 26.4, 20.1, 13.8 ppm; MS (ESI pos.): m/z (%) = 925.64 (12) ($[2M+H]^+$, calcd. 925.59), 463.21 (100) ($[M+H]^+$, calcd. 463.30), 241.23 (25) ($[M-Fmoc+H]^+$, calcd. 241.23), 179.21 (11) ($[M_{fr.}+H]^+$, calcd. 179.08).

5.1.75 (*S*)-(9*H*-Fluoren-9-yl)methyl (4-cyclohexyl-1-(hexylamino)-1-oxobutan-2-yl)carbamate (76).

Compound **76** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and hexan-1-amine (190 μ L, 1.47 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc, 4:1 $\rightarrow$ 1:1) to afford 396 mg (0.81 mmol, 66%) of **76**. TLC: R_F = 0.87 (SiO₂, cyclohexane/EtOAc, 1:1); ¹H-NMR (500 MHz, CDCl₃, 300 K): δ = 7.76 (d, ³*J* = 7.5 Hz, 2H), 7.57 (d, ³*J* = 7.5 Hz), 7.40 (t, ³*J* = 7.4 Hz, 2H), 7.30 (t, ³*J* = 7.4 Hz, 2H), 6.05 (s, 1H), 5.45 (d, ³*J* = 7.5 Hz, 1H), 4.46–4.31 (m, 2H), 4.20 (t, ³*J* = 6.9 Hz, 1H), 4.13–4.02 (m, 1H), 3.33–3.13 (m, 2H), 1.92–1.76 (m, 1H), 1.73–1.57 (m, 6H), 1.53–1.40 (m, 2H), 1.35–1.05 (m, 12H), 0.94–0.79 (m, 5H) ppm; ¹³C-NMR (126 MHz, CDCl₃, 300 K): δ = 171.7, 156.3, 143.9, 141.4, 127.9, 127.2, 125.2, 120.1, 67.1, 55.5, 47.3, 39.7, 37.6, 33.4, 33.3, 33.1, 31.5, 30.4, 29.6, 26.7 (2x), 26.4, 22.7, 14.1 ppm; MS (ESI pos.): m/z (%) = 513.30 (18) ($[M+Na]^+$, calcd. 513.31), 491.33 (100) ($[M+H]^+$, calcd. 491.33), 269.33 (27) ($[M-Fmoc+H]^+$, calcd. 269.25), 179.30 (20) ($[M_{fr.}+H]^+$, calcd. 179.29).

5.1.76 (*S*)-(9*H*-Fluoren-9-yl)methyl (4-cyclohexyl-1-(decylamino)-1-oxobutan-2-yl)carbamate (77).

Compound **77** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and decan-1-amine (290 μ L, 1.47 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc, 4:1 $\rightarrow$ 1:1) to afford 565 mg (1.03 mmol, 84%) of **77**. TLC: R_F = 0.86 (SiO₂, cyclohexane/EtOAc, 1:1); ¹H-NMR (500 MHz, CDCl₃, 300 K): δ = 7.76 (d, ³*J* = 7.5 Hz, 2H), 7.57 (d, ³*J* = 7.5 Hz, 2H), 7.40 (t, ³*J* = 7.4 Hz, 2H), 7.30 (t, ³*J* = 7.4 Hz, 2H), 6.00 (s, 1H), 5.42 (d, ³*J* = 7.5 Hz, 1H), 4.44–4.33 (m, 2H), 4.21 (t, ³*J* = 6.9 Hz, 1H), 4.10–4.01 (m, 1H), 3.32–3.14 (m, 2H), 1.90–1.96 (m, 1H), 1.73–1.58 (m, 6H), 1.51–

1.40 (m, 2H), 1.33–1.07 (m, 20H), 0.93–0.80 (m, 5H) ppm; ^{13}C -NMR (126 MHz, CDCl_3 , 300 K): δ = 171.7, 156.3, 143.9, 141.4, 127.9, 127.2, 125.2, 120.1, 67.1, 55.5, 47.3, 39.7, 37.6, 33.4, 33.3, 33.1, 32.0, 30.4, 29.7 (2x), 29.6, 29.4 (2x), 27.0, 26.7, 26.4, 22.8, 14.3 ppm; MS (ESI pos.): m/z (%) = 569.32 (35) ($[\text{M}+\text{Na}]^+$, calcd. 569.37), 547.34 (100) ($[\text{M}+\text{H}]^+$, calcd. 547.39), 325.26 (11) ($[\text{M}-\text{Fmoc}+\text{H}]^+$, calcd. 325.33).

5.1.77 (9H-Fluoren-9-yl)methyl ((S)-1-((SR)-sec-butylamino)-4-cyclohexyl-1-oxobutan-2-yl)carbamate (78).

Compound **78** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and (*rac*)-butan-2-amine (160 μL , 1.47 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc, 4:1 $\rightarrow$ 1:1) to afford 363 mg (0.79 mmol, 64%) of **78** as a colorless solid. TLC: R_F = 0.76 (SiO_2 , cyclohexane/EtOAc, 1:1); ^1H -NMR (500 MHz, CDCl_3 , 300 K): δ = 7.76 (d, 3J = 7.5 Hz, 2H), 7.58 (d, 3J = 7.5 Hz, 2H), 7.40 (t, 3J = 7.4 Hz, 2H), 7.30 (t, 3J = 7.4 Hz, 2H), 5.86–5.74 (m, 1H), 5.52–5.41 (m, 1H), 4.43–4.32 (m, 2H), 4.21 (t, 3J = 7.1 Hz, 1H), 4.12–4.00 (m, 1H), 3.95–3.84 (m, 1H), 1.90–1.76 (m, 1H), 1.74–1.57 (m, 6H), 1.50–1.38 (m, 2H), 1.30–1.04 (m, 9H), 0.94–0.79 (m, 5H) ppm; ^{13}C -NMR (126 MHz, CDCl_3 , 300 K): δ = 171.7, 156.3, 143.9, 141.4, 127.9, 127.2, 125.2, 120.1, 67.1, 55.5, 47.2, 37.6, 33.4, 33.3, 33.1, 30.6, 30.4, 29.7, 26.7, 26.4, 20.6, 20.5, 10.5 ppm; MS (ESI pos.): m/z (%) = 925.68 (12) ($[2\text{M}+\text{H}]^+$, calcd. 925.59), 463.25 (100) ($[\text{M}+\text{H}]^+$, calcd. 463.30), 241.23 (25) ($[\text{M}-\text{Fmoc}+\text{H}]^+$, calcd. 241.23), 179.15 (9) ($[\text{M}_{\text{fr.}}+\text{H}]^+$, calcd. 179.08).

5.1.78 (S)-(9H-Fluoren-9-yl)methyl (4-cyclohexyl-1-oxo-1-(pentan-3-ylamino)butan-2-yl)carbamate (79).

Compound **79** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and pentan-3-amine (170 μL , 1.47 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc, 4:1 $\rightarrow$ 1:1) to afford 458 mg (0.96 mmol, 78%) of **79**. TLC: R_F = 0.82 (SiO_2 , cyclohexane/EtOAc, 1:1);

¹H-NMR (500 MHz, CDCl₃, 300 K): δ = 7.76 (d, ³*J* = 7.5 Hz, 2H), 7.58 (d, ³*J* = 7.5 Hz, 2H), 7.40 (t, ³*J* = 7.5 Hz, 2H), 7.30 (t, ³*J* = 7.5 Hz, 2H), 5.75 (d, ³*J* = 8.5 Hz, 1H), 5.49 (d, ³*J* = 7.9 Hz, 1H), 4.42–4.32 (m, 2H), 4.21 (t, ³*J* = 7.0 Hz, 1H), 4.14–4.06 (m, 1H), 3.81–3.71 (m, 1H), 1.91–1.78 (m, 1H), 1.76–1.60 (m, 6H), 1.59–1.46 (m, 2H), 1.40–1.30 (m, 2H), 1.29–1.07 (m, 6H), 0.94–0.78 (m, 8H) ppm; ¹³C-NMR (126 MHz, CDCl₃, 300 K): δ = 171.5, 156.3, 143.9, 141.4, 127.9, 127.2, 125.2, 120.1, 67.1, 55.5, 52.3, 47.2, 37.6, 33.4, 33.3, 33.1, 30.5, 26.7, 26.4, 10.4 ppm; MS (ESI pos.): *m/z* (%) = 499.21 (9) ([M+Na]⁺, calcd. 499.29), 477.25 (100) ([M+H]⁺, calcd. 477.31), 255.28 (69) ([M-Fmoc+H]⁺, calcd. 255.23).

5.1.79 (9*H*-Fluoren-9-yl)methyl ((*S*)-4-cyclohexyl-1-(((*SR*)-1-hydroxybutan-2-yl)amino)-1-oxobutan-2-yl)carbamate (80**).**

Compound **80** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and (*rac*)-2-aminobutan-1-ol (133 mg, 1.47 mmol). The crude product was triturated with cyclohexane/CH₂Cl₂ (1:1) to afford 345 mg (0.72 mmol, 59%) of **80**. TLC: *R*_F = 0.30 (SiO₂, cyclohexane/EtOAc, 1:1); ¹H-NMR (400 MHz, DMSO-d₆, 300 K): (mixture of diastereomers) δ = 7.95–7.84 (m, 3H), 7.77–7.69 (m, 2H), 7.95–7.84 (m, 3H), 7.36–7.28 (m, 2H), 4.32–4.17 (m, 3H), 4.03–3.91 (m, 1H), 3.90–3.76 (m, 1H), 3.66–3.51 (m, 2H), 1.72–1.49 (m, 8H), 1.48–1.37 (m, 1H), 1.27–1.04 (m, 6H), 0.90–0.77 (m, 5H) ppm; ¹³C-NMR (101 MHz, DMSO-d₆, 300 K): (mixture of diastereomers) δ = 172.0, 155.8, 143.9, 143.8, 140.7, 127.6, 127.0, 125.3, 120.1, 65.5, 55.0, 54.9, 51.4, 51.2, 47.2 (2x), 36.8, 36.7, 32.9 (2x), 32.7, 29.7, 29.6, 26.1, 25.7, 24.3, 24.1, 10.2 (2x) ppm; MS (ESI pos.): *m/z* (%) = 501.26 (100) ([M+Na]⁺, calcd. 501.27), 479.29 (95) ([M+H]⁺, calcd. 479.29).

5.1.80 (*S*)-(9*H*-Fluoren-9-yl)methyl (4-cyclohexyl-1-(isopentylamino)-1-oxobutan-2-yl)carbamate (81**).**

Compound **81** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and 3-methylbutan-1-amine

(171 μ L, 1.47 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc, 4:1 $\rightarrow$ 2:1) to afford 498 mg (1.05 mmol, 85%) of **81**. TLC: R_F = 0.30 (SiO₂, cyclohexane/EtOAc, 1:1); ¹H-NMR (500 MHz, CDCl₃, 300 K): δ = 7.76 (d, ³ J = 7.6 Hz, 2H), 7.57 (d, ³ J = 7.6 Hz, 2H), 7.40 (t, ³ J = 7.6 Hz, 2H), 7.30 (t, ³ J = 7.6 Hz, 2H), 6.03 (s, 1H), 5.44 (d, ³ J = 8.0 Hz, 1H), 4.45–4.31 (m, 2H), 4.20 (t, ³ J = 6.8 Hz, 1H), 4.11–4.02 (m, 1H), 3.34–3.16 (m, 2H), 1.91–1.77 (m, 1H), 1.73–1.52 (m, 7H), 1.42–1.31 (m, 2H), 1.28–1.06 (m, 6H), 0.94–0.79 (m, 8H) ppm; ¹³C-NMR (126 MHz, CDCl₃, 300 K): δ = 171.7, 156.3, 143.9, 141.4, 127.9, 127.2, 125.2, 120.2, 67.1, 55.5, 47.2, 38.5, 38.0, 37.6, 33.4, 33.3, 33.1, 30.4, 26.7, 26.4, 25.9, 22.5 (2x) ppm; MS (ESI pos.): m/z (%) = 499.21 (37) ([M+Na]⁺, calcd. 499.29), 477.26 (100) ([M+H]⁺, calcd. 477.31), 186.20 (97) ([M_{fr.}]⁺, calcd. 186.10).

5.1.81 (*S*)-(9*H*-Fluoren-9-yl)methyl (4-cyclohexyl-1-(cyclohexylamino)-1-oxobutan-2-yl)carbamate (**82**).

Compound **82** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and cyclohexanamine (170 μ L, 1.47 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc, 4:1 $\rightarrow$ 1:1) to afford 513 mg (1.05 mmol, 86%) of **82** as a colorless solid. TLC: R_F = 0.29 (SiO₂, cyclohexane/EtOAc, 4:1); ¹H-NMR (500 MHz, CDCl₃, 300 K): (mixture of diastereomers) δ = 7.76 (d, ³ J = 7.5 Hz, 2H), 7.58 (d, ³ J = 7.5 Hz, 2H), 7.40 (t, ³ J = 7.5 Hz, 2H), 7.30 (t, ³ J = 7.5 Hz, 2H), 5.90 (d, ³ J = 7.2 Hz, 1H), 5.47 (d, ³ J = 7.8 Hz, 1H), 4.43–4.31 (m, 2H), 4.21 (t, ³ J = 7.1 Hz, 1H), 4.10–4.01 (m, 1H), 3.80–3.70 (m, 1H), 1.93–1.77 (m, 3H), 1.74–1.54 (m, 10H), 1.40–1.28 (m, 2H), 1.27–1.04 (m, 9H), 0.95–0.78 (m, 2H) ppm; ¹³C-NMR (126 MHz, CDCl₃, 300 K): (mixture of diastereomers) δ = 170.7, 156.3, 143.9 (2x), 141.4, 127.9, 127.2, 125.2 (2x), 120.1 (2x), 67.1, 55.5, 48.4, 47.2, 37.6, 33.4, 33.3, 33.2, 33.0, 30.6, 26.7, 26.4, 25.6, 24.9 ppm; MS (ESI pos.): m/z (%) = 511.24 (100) ([M+Na]⁺, calcd. 511.65).

5.1.82 (*S*)-(9*H*-Fluoren-9-yl)methyl (4-cyclohexyl-1-morpholino-1-oxobutan-2-yl)carbamate (**83**).

Compound **83** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and morpholine (128 mg, 1.47 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 4:1 → 1:1 → 1:3) to afford 613 mg (1.07 mmol, 87%) of **83** as a colorless solid. TLC: R_F = 0.55 (SiO₂, cyclohexane/EtOAc, 1:1); ¹H-NMR (500 MHz, CDCl₃, 300 K): δ = 7.76 (d, ³*J* = 7.5 Hz, 2H), 7.65–7.53 (m, 2H), 7.40 (t, ³*J* = 7.4 Hz, 2H), 7.31 (t, ³*J* = 7.5 Hz, 2H), 5.70 (d, ³*J* = 8.5 Hz, 1H), 4.65–4.56 (m, 1H), 4.42–4.31 (m, 2H), 4.22 (t, ³*J* = 7.1 Hz, 1H), 3.75–3.45 (m, 8H), 1.78–1.51 (m, 7H), 1.34–1.07 (m, 6H), 0.95–0.81 (m, 2H) ppm; ¹³C-NMR (126 MHz, CDCl₃, 300 K): δ = 170.7, 156.1, 144.1, 143.9, 141.4, 127.8, 127.2, 125.3, 120.1, 67.1, 67.0, 66.8, 50.8, 47.3, 46.2, 42.6, 37.7, 33.5, 33.3, 32.6, 30.8, 26.7, 26.4 (2x) ppm; MS (ESI pos.): *m/z* (%) = 499.26 (100) ([M+Na]⁺, calcd. 499.26), 477.31 (65) ([M+H]⁺, calcd. 477.28), 517.36 (23) ([M+K]⁺, calcd. 515.28).

5.1.83 (*S*)-(9*H*-Fluoren-9-yl)methyl (4-cyclohexyl-1-((4-fluorophenyl)amino)-1-oxobutan-2-yl)carbamate (**84**).

Compound **84** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 400 mg, 0.98 mmol) and 4-fluoroaniline (112 μ L, 1.18 mmol). The crude product was recrystallized from acetone to afford 438 mg (0.88 mmol, 89%) of **84** as a colorless solid. TLC: R_F = 0.26 (SiO₂, cyclohexane/EtOAc, 4:1); ¹H-NMR (600 MHz, DMSO-*d*₆, 300 K): δ = 10.06 (s, 1H), 7.89 (d, ³*J* = 7.5 Hz, 2H), 7.74 (t, ³*J* = 7.8 Hz, 2H), 7.66–7.60 (m, 3H), 7.41 (td, ³*J* = 7.5, 3.3 Hz, 2H), 7.32 (td, ³*J* = 7.5, 3.2 Hz, 2H), 7.14 (t, ³*J* = 8.9 Hz, 2H), 4.32–4.18 (m, 3H), 4.12–4.02 (m, 1H), 1.75–1.55 (m, 7H), 1.33–1.26 (m, 1H), 1.25–1.07 (m, 5 H), 0.90–0.79 (m, 2H) ppm; ¹³C-NMR (150 MHz, DMSO-*d*₆, 300 K): δ = 171.0, 158.0 (¹*J*_{CF} = 241.9 Hz), 156.0, 143.8, 143.7, 140.7, 135.3, 127.6, 127.0, 125.3, 120.9, 120.0 (³*J*_{CF} = 8.1 Hz), 115.2 (²*J*_{CF} = 22.3 Hz), 65.6, 55.6, 46.6, 36.8, 33.1, 32.8, 32.6, 29.3, 26.1, 25.7 (2x) ppm; MS (ESI pos.): *m/z* (%) = 523.23 (52) ([M+Na]⁺, calcd. 523.24), 501.27 (47) ([M+H]⁺, calcd. 501.26), 279.25 (25) ([M-Fmoc+H]⁺, calcd. 279.25), 179.28 (100) ([M_{fr.}+H]⁺, calcd. 179.29).

5.1.84 (*S*)-(9*H*-Fluoren-9-yl)methyl (1-((4-chlorophenyl)amino)-4-cyclohexyl-1-oxobutan-2-yl)carbamate (**85**).

Compound **85** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 400 mg, 0.98 mmol) and 4-chloroaniline (150 mg, 1.18 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc, 4:1 → 1:1 → 1:3) to afford 439 mg (0.85 mmol, 86%) of **85** as a colorless solid. TLC: R_F = 0.87 (SiO₂, cyclohexane/EtOAc, 1:1); ¹H-NMR (250 MHz, DMSO-*d*₆, 300 K): δ = 10.14 (s, 1H), 7.89 (d, ³*J* = 7.4 Hz, 2H), 7.74–7.59 (m, 5H), 7.46–7.26 (m, 6H), 4.32–4.17 (m, 3H), 4.15–4.03 (m, 1H), 1.78–1.52 (m, 7H), 1.35–1.04 (m, 6H), 0.96–0.75 (m, 2H) ppm; ¹³C-NMR (126 MHz, CDCl₃, 300 K): δ = 170.2, 156.8, 143.9, 141.4, 136.2, 129.6, 129.1, 128.0, 127.3, 125.1, 121.4, 120.2, 67.4, 56.2, 47.2, 37.6, 33.4, 33.3, 29.8, 29.6, 27.1, 26.6, 26.4 ppm; MS (ESI pos.): *m/z* (%) = 539.23 (58) ([M+Na]⁺, calcd. 539.21), 517.26 (100) ([M+H]⁺, calcd. 517.23), 295.21 (23) ([M-Fmoc+H]⁺, calcd. 295.16), 179.29 (95) ([M_{fr}+H]⁺, calcd. 179.09).

5.1.85 (*S*)-(9*H*-Fluoren-9-yl)methyl (1-((4-bromophenyl)amino)-4-cyclohexyl-1-oxobutan-2-yl)carbamate (**86**).

Compound **86** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 400 mg, 0.98 mmol) and 4-bromoaniline (203 mg, 1.18 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 4:1 → 1:1 → 1:3 → 1:20) and further recrystallized from acetone to afford 487 mg (0.87 mmol, 88%) of **86** as a colorless solid. TLC: R_F = 0.36 (SiO₂, cyclohexane/EtOAc, 4:1); ¹H-NMR (600 MHz, DMSO-*d*₆, 300 K): δ = 10.15 (s, 1H), 7.89 (d, ³*J* = 7.6 Hz, 2H), 7.74 (t, ³*J* = 7.9 Hz, 2H), 7.66 (d, ³*J* = 7.9 Hz, 1H), 7.58 (d, ³*J* = 8.7 Hz, 2H), 7.48 (d, ³*J* = 8.7 Hz, 2H), 7.44–7.38 (m, 2H), 7.34–7.29 (m, 2H), 4.32–4.20 (m, 3H), 4.12–4.06 (m, 1H), 1.76–1.56 (m, 7H), 1.33–1.25 (m, 1H), 1.24–1.06 (m, 5H), 0.91–0.80 (m, 2H) ppm; ¹³C-NMR (150 MHz, DMSO-*d*₆, 300 K): δ = 170.3, 155.0, 142.8, 142.7, 139.7, **137.3**, 130.5, 126.6, 126.0, 124.3, 120.1, 119.0, 113.8, 64.6, 54.7, 45.6, 35.8, 32.1, 31.8, 31.6, 28.2, 25.1, 24.7 (2x) ppm; MS

(ESI pos.): m/z (%) = 563.17 (16) ($[M+H]^+$, calcd. 563.18), 561.17 (15) ($[M+H]^+$, calcd. 561.18), 179.29 (100) ($[M_{fr}+H]^+$, calcd. 179.09).

5.1.86 (S)-(9H-Fluoren-9-yl)methyl (4-cyclohexyl-1-((3,5-difluorophenyl)amino)-1-oxobutan-2-yl)carbamate (87).

Compound **87** was prepared according to general procedure E using (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 400 mg, 0.98 mmol) and 3,5-difluoroaniline (152 mg, 1.18 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 4:1 $\rightarrow$ 1:1 $\rightarrow$ 1:3 $\rightarrow$ 1:20) and further recrystallized from acetone to afford 410 mg (0.79 mmol, 81%) of **87** as a colorless solid. TLC: R_F = 0.37 (SiO₂, cyclohexane/EtOAc, 4:1); ¹H-NMR (600 MHz, DMSO-d₆, 300 K): δ = 10.41 (s, 1H), 7.89 (d, ³ J = 7.4 Hz, 2H), 7.76–7.69 (m, 3H), 7.44–7.39 (m, 2H), 7.37–7.29 (m, 4H), 6.96–6.86 (m, 1H), 4.33–4.17 (m, 3H), 4.10–4.03 (m, 1H), 1.75–1.54 (m, 7H), 1.35–1.25 (m, 1H), 1.24–1.05 (m, 5H), 0.91–0.80 (m, 2H) ppm; ¹³C-NMR (150 MHz, DMSO-d₆, 300 K): δ = 172.5, 171.9, 162.4 (¹ J_{CF} = 243.5 Hz), 162.3 (¹ J_{CF} = 243.5 Hz), 156.1, 143.8, 143.7, 141.5, 141.4, 140.7, 127.6, 127.0, 125.2, 120.0, 102.0 (² J_{CF} = 28.9 Hz), 98.5 (² J_{CF} = 28.9 Hz), 65.6, 55.8, 46.6, 36.8, 33.1, 32.8, 32.6, 29.0, 26.1, 25.7 (2x) ppm; MS (ESI pos.): m/z (%) = 541.21 (16) ($[M+Na]^+$, calcd. 541.23), 519.18 (22) ($[M+H]^+$, calcd. 519.25), 297.20 (11) ($[M-Fmoc+H]^+$, calcd. 297.18), 179.30 (100) ($[M_{fr}+H]^+$, calcd. 179.09).

5.1.87 (S)-(9H-Fluoren-9-yl)methyl (4-cyclohexyl-1-((3,5-dichlorophenyl)amino)-1-oxobutan-2-yl)carbamate (88).

Compound **88** was prepared according to general procedure E using (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 400 mg, 0.98 mmol) and 3,5-dichloroaniline (191 mg, 1.18 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 4:1 $\rightarrow$ 1:1 $\rightarrow$ 1:3) and further recrystallized from MeOH to afford 285 mg (0.55 mmol, 56%) of **88** as a colorless solid. TLC: R_F = 0.45 (SiO₂, cyclohexane/EtOAc, 4:1); ¹H-NMR (500 MHz, DMSO-d₆, 300 K): δ = 10.38 (s, 1H), 7.89 (d, ³ J = 7.6 Hz, 2H), 7.76–7.71 (m, 3H), 7.69 (d, ⁴ J = 1.8 Hz, 2H), 7.41

(t, $^3J = 7.5$ Hz, 2H), 7.32 (t, $^3J = 7.5$ Hz, 2H), 7.28 (t, $^4J = 1.7$ Hz, 1H), 4.33–4.12 (m, 3H), 4.08–4.01 (m, 1H), 1.76–1.55 (m, 7H), 1.34–1.24 (m, 1H), 1.23–1.05 (m, 5H), 0.90–0.78 (m, 2H) ppm; ^{13}C -NMR (126 MHz, DMSO- d_6 , 300 K): $\delta = 172.0, 156.1, 143.8$ (2x), 141.2, 140.7, 134.1, 127.6, 127.0, 125.3 (2x), 122.5, 120.1 (2x), 117.3, 65.6, 55.9, 46.7, 36.8, 33.2, 32.9, 32.6, 29.0, 26.1, 25.8 (2x) ppm; MS (ESI pos.): m/z (%) = 573.17 (12) ($[\text{M}+\text{Na}]^+$, calcd. 573.13), 551.20 (18) ($[\text{M}+\text{H}]^+$, calcd. 551.19), 179.17 (100) ($[\text{M}_{\text{fr.}}+\text{H}]^+$, calcd. 179.09).

5.1.88 (*S*)-(9*H*-Fluoren-9-yl)methyl (4-cyclohexyl-1-((3,5-dibromophenyl)amino)-1-oxobutan-2-yl)carbamate (89).

Compound **89** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 400 mg, 0.98 mmol) and 3,5-dibromoaniline (296 mg, 1.18 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 4:1 $\rightarrow$ 1:1) to afford 404 mg (0.63 mmol, 64%) of **89** as a colorless solid. TLC: $R_F = 0.43$ (SiO_2 , cyclohexane/EtOAc, 4:1); ^1H -NMR (600 MHz, DMSO- d_6 , 300 K): $\delta = 10.31$ (s, 1H), 7.91–7.85 (m, 4H), 7.76–7.68 (m, 3H), 7.50–7.48 (m, 1H), 7.43–7.39 (m, 2H), 7.32 (t, $^3J = 7.4$ Hz, 2H), 4.34–4.19 (m, 3H), 4.07–4.00 (m, 1H), 1.75–1.56 (m, 7H), 1.33–1.25 (m, 1H), 1.23–1.06 (m, 5H), 0.90–0.80 (m, 2H) ppm; ^{13}C -NMR (150 MHz, DMSO- d_6 , 300 K): $\delta = 171.9, 156.1, 143.8, 143.7, 141.5, 141.4, 140.7, 127.8, 127.6, 127.0, 125.2, 122.3, 120.5, 120.0, 65.6, 55.9, 46.6, 36.8, 33.1, 32.8, 32.6, 28.9, 26.1, 25.7$ (2x) ppm; MS (ESI neg.): m/z (%) = 675.20 (8) ($[\text{M}+\text{Cl}]^-$, calcd. 675.53), 417.23 (100) ($[\text{M}-\text{Fmoc}+\text{H}]^-$, calcd. 417.00).

5.1.89 (*S*)-(9*H*-Fluoren-9-yl)methyl (1-(benzylamino)-4-cyclohexyl-1-oxobutan-2-yl)carbamate (90).

Compound **90** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 400 mg, 0.98 mmol) and phenylmethanamine (126 mg, 1.18 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc, 1:4 $\rightarrow$ EtOH) to afford 373 mg (0.75 mmol, 76%) of **90** as a colorless solid. TLC: $R_F = 0.83$

(SiO₂, cyclohexane/EtOAc, 1:3); ¹H-NMR (500 MHz, DMSO-d₆, 300 K): δ = 8.41 (t, ³J = 5.9 Hz, 1H), 7.89 (d, ³J = 7.6 Hz, 2H), 7.77–7.70 (m, 2H), 7.50 (d, ³J = 8.2 Hz, 1H), 7.42 (t, ³J = 7.4 Hz, 2H), 7.35–7.18 (m, 7H), 4.37–4.16 (m, 5H), 4.02–3.94 (m, 1H), 1.73–1.50 (m, 7H), 1.28–1.05 (m, 6H), 0.90–0.75 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-d₆, 300 K): δ = 172.0, 156.0, 143.9, 143.8, 140.7, 139.5, 128.2, 127.6, 127.1, 127.0, 126.7, 125.3, 120.1, 65.6, 55.0, 46.7, 42.0, 36.7, 33.0, 32.0, 32.7, 29.4, 26.2, 25.8 (2x) ppm; MS (ESI pos.): *m/z* (%) = 519.23 (100) ([M+Na]⁺, calcd. 519.26), 520.24 (34) ([M+Na]⁺, calcd. 520.28).

5.1.90 (*S*)-(9*H*-Fluoren-9-yl)methyl (1-((4-chlorobenzyl)amino)-4-cyclohexyl-1-oxobutan-2-yl)carbamate (**91**).

Compound **91** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 400 mg, 0.98 mmol) and (4-chlorophenyl)methanamine (167 mg, 1.18 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc, 8:1 → 1:1 → EtOAc) to afford 350 mg (0.66 mmol, 67%) of **91** as a colorless solid. TLC: *R_F* = 0.51 (SiO₂, cyclohexane/EtOAc, 8:1); ¹H-NMR (500 MHz, DMSO-d₆, 300 K): δ = 8.45 (t, ³J = 5.7 Hz, 1H), 7.89 (d, ³J = 7.7 Hz, 2H), 7.77–7.70 (m, 2H), 7.50 (d, ³J = 8.0 Hz, 1H), 7.41 (t, ³J = 7.4 Hz, 2H), 7.38–7.22 (m, 6H), 4.35–4.13 (m, 5H), 3.99–3.92 (m, 1H), 1.72–1.47 (m, 7H), 1.25–1.02 (m, 6H), 0.89–0.75 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-d₆, 300 K): δ = 172.1, 156.0, 143.9, 143.8, 140.7, 138.6, 131.3, 129.0, 128.1, 127.6, 127.1, 127.0, 125.3, 120.1, 65.6, 55.0, 46.7, 41.4, 36.7, 33.0, 32.9, 32.6, 29.4, 26.2, 25.8 (2x) ppm; MS (ESI pos.): *m/z* (%) = 553.21 (33) ([M+Na]⁺, calcd. 553.22), 531.25 (100) ([M+H]⁺, calcd. 531.24), 309.17 (35) ([M-Fmoc+H]⁺, calcd. 309.18), 179.29 (69) ([M_{fr}+H]⁺, calcd. 179.29).

5.1.91 (*S*)-(9*H*-Fluoren-9-yl)methyl (4-cyclohexyl-1-oxo-1-((pyridin-4-ylmethyl)amino)butan-2-yl)carbamate (**92**).

Compound **92** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and pyridin-4-ylmethanamine

(159 mg, 1.47 mmol). The crude product was triturated with cyclohexane/CH₂Cl₂ (1:1) to afford 501 mg (1.01 mmol, 82%) of **92** as a colorless solid. TLC: R_F = 0.27 (SiO₂, cyclohexane/EtOAc, 1:20); ¹H-NMR (500 MHz, DMSO-d₆, 300 K): δ = 8.54 (t, ³ J = 5.9 Hz, 1H), 8.46 (d, ³ J = 5.7 Hz, 2H), 7.89 (d, ³ J = 7.6 Hz, 2H), 7.77–7.71 (m, 2H), 7.57 (d, ³ J = 8.3 Hz, 1H), 7.41 (t, ³ J = 7.5 Hz, 2H), 7.31 (t, ³ J = 7.4 Hz, 2H), 7.23 (d, ³ J = 5.7 Hz, 2H), 4.36–4.18 (m, 5H), 4.02–3.94 (m, 1H), 1.75–1.51 (m, 7H), 1.27–1.05 (m, 6), 0.90–0.76 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-d₆, 300 K): δ = 172.4, 156.1, 149.4, 148.5, 143.9, 143.8, 140.7 (2x), 127.6, 127.0, 125.3, 122.1, 120.1, 65.6, 55.0, 46.7, 41.1, 36.7, 33.1, 32.9, 32.7, 29.2, 26.4, 26.2, 25.8 (2x) ppm; MS (ESI pos.): m/z (%) = 498.29 (100) ([M+H]⁺, calcd. 498.28).

5.1.92 (S)-(9H-Fluoren-9-yl)methyl (4-cyclohexyl-1-oxo-1-((pyridin-3-ylmethyl)amino)butan-2-yl)carbamate (93).

Compound **93** was prepared according to general procedure E using (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and pyridin-3-ylmethanamine (159 mg, 1.47 mmol). The crude product was triturated with cyclohexane/CH₂Cl₂ (1:1) to afford 384 mg (0.77 mmol, 63%) of **93** as a colorless solid. TLC: R_F = 0.36 (SiO₂, cyclohexane/EtOAc, 1:20); ¹H-NMR (500 MHz, DMSO-d₆, 300 K): δ = 8.50 (t, ³ J = 5.7 Hz, 1H), 8.47 (s, 1H), 8.46–8.40 (m, 1H), 7.89 (d, ³ J = 7.6 Hz, 2H), 7.77–7.70 (m, 2H), 7.63 (d, ³ J = 7.9 Hz, 1H), 7.53 (d, ³ J = 8.0 Hz, 1H), 7.41 (t, ³ J = 7.5 Hz, 2H), 7.36–7.26 (m, 3H), 4.37–4.14 (m, 5H), 3.99–3.90 (m, 1H), 1.72–1.47 (m, 7H), 1.25–1.04 (m, 6H), 0.88–0.74 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-d₆, 300 K): δ = 172.2, 156.0, 148.7, 148.0, 143.9, 143.8, 140.7 (2x), 135.0, 134.9, 127.6, 127.0, 125.4, 125.3, 123.4, 120.1, 65.6, 55.0, 46.7, 36.7, 33.0, 32.9, 32.6, 29.3, 26.3, 26.2, 25.8, 25.7 ppm; MS (ESI pos.): m/z (%) = 498.30 (100) ([M+H]⁺, calcd. 498.28).

5.1.93 (S)-(9H-Fluoren-9-yl)methyl (4-cyclohexyl-1-oxo-1-((pyridin-2-ylmethyl)amino)butan-2-yl)carbamate (94).

Compound **94** was prepared according to general procedure E using (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and pyridin-2-ylmethanamine

(159 mg, 1.47 mmol). The crude product was triturated with cyclohexane/CH₂Cl₂ (1:1) to afford 495 mg (1.00 mmol, 81%) of **94** as a colorless solid. TLC: R_F = 0.31 (SiO₂, cyclohexane/EtOAc, 1:3); ¹H-NMR (500 MHz, DMSO-d₆, 300 K): δ = 8.50 (t, ³ J = 5.8 Hz, 1H), 8.48–8.45 (m, 1H), 7.89 (d, ³ J = 7.6 Hz, 2H), 7.77–7.68 (m, 3H), 7.55 (d, ³ J = 8.1 Hz, 1H), 7.41 (t, ³ J = 7.5 Hz, 2H), 7.35–7.20 (m, 4H), 4.37 (d, ³ J = 5.6 Hz, 2H), 4.34–4.18 (m, 3H), 4.04–3.96 (m, 1H), 1.76–1.50 (m, 7H), 1.28–1.05 (m, 6H), 0.90–0.77 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-d₆, 300 K): δ = 172.3, 158.5, 156.0, 148.7, 143.9, 143.8, 140.7, 136.6, 127.6, 127.0, 125.3, 122.1, 120.8, 120.1, 65.6, 55.0, 46.7, 44.1, 36.8, 33.1, 32.9, 32.7, 29.3, 26.2, 25.8 (2x) ppm; MS (ESI pos.): m/z (%) = 498.29 (100) ([M+H]⁺, calcd. 498.28).

5.1.94 (S)-(9H-Fluoren-9-yl)methyl (4-cyclohexyl-1-oxo-1-((thiophen-2-ylmethyl)amino)butan-2-yl)carbamate (95).

Compound **95** was prepared according to general procedure E using (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and thiophen-2-ylmethanamine (113 mg, 1.47 mmol). The crude product was triturated with cyclohexane/CH₂Cl₂ (1:1) to afford 468 mg (0.93 mmol, 76%) of **95**. TLC: R_F = 0.86 (SiO₂, cyclohexane/EtOAc, 1:1); ¹H-NMR (500 MHz, DMSO-d₆, 300 K): δ = 8.51 (t, ³ J = 5.7 Hz, 1H), 7.89 (d, ³ J = 7.6 Hz, 2H), 7.77–7.70 (m, 2H), 7.49 (d, ³ J = 8.3 Hz, 1H), 7.42 (t, ³ J = 7.4 Hz, 2H), 7.38–7.35 (m, 1H), 7.32 (t, ³ J = 7.4 Hz, 2H), 6.98–6.90 (m, 2H), 4.59–4.34 (m, 2H), 4.33–4.15 (m, 3H), 4.00–3.90 (m, 1H), 1.71–1.47 (m, 7H), 1.27–1.04 (m, 6H), 0.89–0.76 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-d₆, 300 K): δ = 171.8, 155.9, 143.9, 143.8, 142.5, 140.7, 127.6, 127.0, 126.6, 125.4, 125.2, 125.0, 120.1, 65.6, 54.8, 46.7, 37.2, 36.7, 33.0, 32.9, 32.6, 29.4, 26.2, 25.8 (2x) ppm; MS (ESI pos.): m/z (%) = 525.22 (100) ([M+Na]⁺, calcd. 525.22).

5.1.95 (S)-(9H-Fluoren-9-yl)methyl (1-((4-chlorophenethyl)amino)-4-cyclohexyl-1-oxobutan-2-yl)carbamate (96).

Compound **96** was prepared according to general procedure E using (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and 2-(4-chlorophenyl)ethanamine (229 mg, 1.47 mmol). The crude product was purified by column chromatography on silica

(CH₂Cl₂/MeOH, 10:1) to afford 418 mg (0.77 mmol, 62%) of **96** as a colorless solid. TLC: *R*_F = 0.89 (SiO₂, cyclohexane/EtOAc, 1:1); ¹H-NMR (500 MHz, CDCl₃, 300 K): δ = 7.76 (d, ³*J* = 7.5 Hz, 2H), 7.56 (d, ³*J* = 7.5 Hz, 2H), 7.40 (t, ³*J* = 7.5 Hz, 2H), 7.30 (t, ³*J* = 7.5 Hz, 2H), 7.22 (d, ³*J* = 8.4 Hz, 2H), 7.07 (d, ³*J* = 8.3 Hz, 2H), 5.95 (s, 1H), 5.24 (d, ³*J* = 7.8 Hz, 1H), 4.47–4.27 (m, 2H), 4.22 (t, ³*J* = 6.9 Hz, 1H), 4.08–3.99 (m, 1H), 3.60–3.30 (m, 2H), 2.83–2.64 (m, 2H), 1.91–1.46 (m, 7H), 1.35–1.02 (m, 6H), 0.95–0.73 (m, 2H); ¹³C-NMR (126 MHz, CDCl₃, 300 K): δ = 171.9, 156.3, 143.9, 141.4, 137.2, 132.5, 130.2, 128.8, 127.9, 127.2, 125.1, 120.1, 67.2, 55.5, 47.3, 40.5, 37.6, 35.1, 33.4, 33.3, 33.1, 30.2, 26.7, 26.4 ppm; MS (ESI pos.): *m/z* (%) = 511.18 (100) (M-Cl+2H)⁺, calcd. 511.30).

5.1.96 (S)-tert-Butyl 4-(2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoyl)piperazine-1-carboxylate (97**).**

Compound **97** was prepared according to general procedure E using (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and a solution of *tert*-butyl piperazine-1-carboxylate (274 mg, 1.47 mmol) in DMF (4 mL) with DIPEA (80 mg, 0.61 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 4:1 → 1:1 → 1:3) to afford 613 mg (1.07 mmol, 87%) of **97**. TLC: *R*_F = 0.73 (SiO₂, cyclohexane/EtOAc, 1:1); ¹H-NMR (500 MHz, CDCl₃, 300 K): δ = 7.76 (d, ³*J* = 7.5 Hz, 2H), 7.64–7.57 (m, 2H), 7.40 (t, ³*J* = 7.5 Hz, 2H), 7.31 (t, ³*J* = 7.5 Hz, 2H), 5.69 (d, ³*J* = 8.5 Hz, 1H), 4.68–4.55 (m, 1H), 4.41–4.31 (m, 2H), 4.22 (t, ³*J* = 7.2 Hz, 1H), 3.71–3.23 (m, 8H), 1.79–1.53 (m, 7H), 1.48 (s, 9H), 1.31–1.04 (m, 6H), 0.90–0.80 (m, 2H) ppm; ¹³C-NMR (126 MHz, CDCl₃, 300 K): δ = 170.8, 156.1, 154.6, 144.0, 143.9, 141.4, 127.8, 127.2, 125.3, 120.1, 80.6, 67.1, 51.0, 47.3, 45.6, 42.1, 37.6, 33.5, 33.2, 32.7, 30.9, 28.5, 26.7, 26.4 ppm; MS (ESI pos.): *m/z* (%) = 520.28 (100) ([M-Boc+2Na]⁺, calcd. 520.26), 598.32 (89) ([M+Na]⁺, calcd. 598.33), 576.35 (21) ([M+H]⁺, calcd. 576.35).

5.1.97 (S)-(9H-Fluoren-9-yl)methyl (1-(4-(4-chlorophenyl)piperazin-1-yl)-4-cyclohexyl-1-oxobutan-2-yl)carbamate (98**).**

Compound **98** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and 1-(4-chlorophenyl)piperazine (290 mg, 1.47 mmol). The crude product was triturated with cyclohexane/CH₂Cl₂ (1:1) to afford 651 mg (1.11 mmol, 91%) of **98** as a colorless solid. TLC: *R*_F = 0.68 (SiO₂, cyclohexane/EtOAc, 1:1); ¹H-NMR (500 MHz, DMSO-*d*₆, 300 K): δ = 7.88 (d, ³*J* = 7.5 Hz, 2H), 7.72 (dd, ³*J* = 7.6, 3.5 Hz, 2H), 7.63 (d, ³*J* = 8.3 Hz, 1H), 7.40 (dt, ³*J* = 7.6, 3.1 Hz, 2H), 7.30 (t, ³*J* = 7.4 Hz, 2H), 7.24 (d, ³*J* = 9.0 Hz, 2H), 6.94 (d, ³*J* = 9.0 Hz, 2H), 4.46–4.36 (m, 1H), 4.32–4.17 (m, 3H), 3.72–3.44 (m, 4H), 3.17–2.99 (m, 4H), 1.71–1.48 (m, 7H), 1.30–1.02 (m, 6H), 0.91–0.75 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆, 300 K): δ = 170.1, 155.9, 149.5, 143.8, 140.7, 128.7, 127.6, 127.0, 125.3, 122.7, 120.1, 117.3, 65.6, 50.7, 48.6, 48.1, 46.7, 44.6, 41.2, 37.0, 32.9, 32.7, 29.0, 26.4, 26.2, 25.8 (2x) ppm; MS (ESI pos.): *m/z* (%) = 586.20 (9) ([M+H]⁺, calcd. 586.29), 364.23 (100) ([M-Fmoc+2H]⁺, calcd. 364.22), 295.22 (93) ([M_{fr.}]⁺, calcd. 295.07).

5.1.98 (*S*)-(9*H*-Fluoren-9-yl)methyl (1-(4-(4-bromophenyl)piperazin-1-yl)-4-cyclohexyl-1-oxobutan-2-yl)carbamate (**99**).

Compound **99** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and 1-(4-bromophenyl)piperazine (355 mg, 1.47 mmol). The crude product was triturated with cyclohexane/CH₂Cl₂ (1:1) to afford 610 mg (0.97 mmol, 79%) of **99**. TLC: *R*_F = 0.79 (SiO₂, cyclohexane/EtOAc, 1:1); ¹H-NMR (500 MHz, DMSO-*d*₆, 300 K): δ = 7.88 (d, ³*J* = 7.6 Hz, 2H), 7.72 (dd, ³*J* = 7.6, 3.4 Hz, 2H), 7.63 (d, ³*J* = 8.3 Hz, 1H), 7.40 (dt, ³*J* = 7.4, 3.0 Hz, 2H), 7.35 (d, ³*J* = 8.9 Hz, 2H), 7.30 (t, ³*J* = 7.4 Hz, 2H), 6.89 (d, ³*J* = 9.1 Hz, 2H), 4.45–4.37 (m, 1H), 4.31–4.17 (m, 3H), 3.69–3.45 (m, 4H), 3.17–2.99 (m, 4H), 1.69–1.48 (m, 7H), 1.28–1.03 (m, 6H), 0.91–0.74 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆, 300 K): δ = 170.1, 155.9, 149.5, 143.8, 140.7, 131.5, 127.6, 127.0, 125.3, 120.1, 117.7, 110.5, 65.6, 50.7, 48.4, 47.9, 46.7, 44.6, 41.2, 37.0, 32.9, 32.7, 29.0, 26.2, 25.8 (2x) ppm; MS (ESI pos.): *m/z* (%) = 632.27 (22) ([M+H]⁺, calcd. 632.23), 408.20 (41) ([M-Fmoc+2H]⁺, calcd. 408.17), 339.11 (100) ([M_{fr.}]⁺, calcd. 339.02).

5.1.99 *tert*-Butyl 4-(benzo[*d*]thiazol-2-yl)piperazine-1-carboxylate (**100**).

2-Chlorobenzo[*d*]thiazole (170 mg, 1.00 mmol) and *tert*-butyl piperazine-1-carboxylate (373 mg, 2.00 mmol) were suspended in water (2 mL) and stirred for 5 d at rt. The precipitate was dissolved in EtOAc, the aqueous phase was extracted 3-times with EtOAc (50 mL) and dried over MgSO₄. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica (cyclohexane/EtOAc 20:1 → EtOAc) to afford 282 mg (0.88 mmol, 88%) of **100** as a colorless solid. TLC: *R*_F = 0.81 (SiO₂, cyclohexane/EtOAc, 1:1); ¹H-NMR (500 MHz, CDCl₃, 300 K): δ = 7.61 (d, ³*J* = 7.9 Hz, 1H), 7.56 (d, ³*J* = 8.0 Hz, 1H), 7.30 (dt, ³*J* = 7.7, 1.3 Hz, 1H), 7.09 (dt, ³*J* = 7.7, 1.2 Hz, 1H), 3.64–3.54 (m, 8H), 1.49 (s, 9H) ppm; ¹³C-NMR (126 MHz, CDCl₃, 300 K): δ = 168.8, 154.7, 152.7, 130.8, 126.2, 121.8, 120.9, 119.4, 80.6, 48.3, 43.4, 28.5 ppm; MS (ESI pos.): *m/z* (%) = 342.18 (65) ([M+Na]⁺, calcd. 342.13), 320.24 (36) ([M+H]⁺, calcd. 320.15), 264.19 (100) ([M-Boc+EtOH]⁺, calcd. 264.12), 220.08 (61) ([M-Boc+H]⁺, calcd. 220.09).

5.1.1002-(Piperazin-1-yl)benzo[*d*]thiazole (**101**).

tert-Butyl 4-(benzo[*d*]thiazol-2-yl)piperazine-1-carboxylate (**100**, 365 mg, 1.14 mmol) was dissolved in CH₂Cl₂ (15 mL), cooled to 0 °C and TFA (2 mL) was added. The mixture was stirred for 3 h at rt and the solvent was removed under reduced pressure. The crude product was dissolved in MeOH and K₂CO₃ (3.6 g, 25.96 mmol) was added. The mixture was stirred for 5 min at 5 °C, filtered, and the solvent was removed under reduced pressure. Water was added, and the aqueous phase was extracted 3-times with EtOAc (50 mL). The organic extracts were combined, dried over MgSO₄, and the solvent was evaporated under reduced pressure to afford 246 mg (1.12 mmol, quant.) of **101**. TLC: *R*_F = 0.51 (SiO₂, cyclohexane/EtOAc, 20:1); ¹H-NMR (500 MHz, DMSO-*d*₆, 300 K): δ = 7.74 (d, ³*J* = 7.9 Hz, 1H), 7.44 (d, ³*J* = 8.1 Hz, 1H), 7.26 (dt, ³*J* = 7.9, 1.3 Hz, 1H), 7.05 (dt, ³*J* = 7.7, 1.1 Hz, 1H), 3.51–3.43 (m, 4H), 2.86–2.74 (m, 4H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆, 300 K): δ = 168.4, 152.2, 130.2, 125.9, 121.1, 121.0, 118.5, 49.3, 45.0 ppm; MS (ESI pos.): *m/z* (%) = 220.10 (100) ([M+H]⁺, calcd. 220.09).

5.1.101 (*S*)-(9*H*-Fluoren-9-yl)methyl (1-(4-(benzo[*d*]thiazol-2-yl)piperazin-1-yl)-4-cyclohexyl-1-oxobutan-2-yl)carbamate (**102**).

Compound **102** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and a solution of 2-(piperazin-1-yl)benzo[*d*]thiazole (**101**, 260 mg, 1.19 mmol, 0.9 eq) in DMF/THF (7 mL, 6:1). The crude product was purified by column chromatography on silica (CH₂Cl₂/MeOH, 10:1) to afford 466 mg (0.77 mmol, 62%) of **102**. TLC: *R*_F = 0.96 (SiO₂, CH₂Cl₂/MeOH, 10:1); ¹H-NMR (400 MHz, DMSO-*d*₆, 300 K): δ = 7.91–7.85 (m, 2H), 7.79 (d, ³*J* = 7.5 Hz, 1H), 7.73 (dd, ³*J* = 7.7, 3.5 Hz, 2H), 7.64 (d, ³*J* = 8.2 Hz, 1H), 7.48 (d, ³*J* = 7.6 Hz, 1H), 7.44–7.40 (m, 2H), 7.35–7.26 (m, 3H), 7.13–7.06 (m, 1H), 4.47–4.38 (m, 1H), 4.32–4.18 (m, 3H), 3.74–3.46 (m, 8H), 1.71–1.51 (m, 7H), 1.27–1.04 (m, 6H), 0.92–0.77 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆, 300 K): δ = 173.3, 168.1, 168.0, 152.3, 143.9, 130.4, 127.3 (2x), 126.0, 124.6, 123.9, 121.4 (2x), 121.2, 120.0, 118.7, 67.8, 50.4, 47.1, 40.9, 37.1, 33.0, 32.9, 32.7, 32.6, 32.1, 26.2, 25.8 (2x) ppm; MS (ESI pos.): *m/z* (%) = 387.24 (100) ([M-Fmoc+H]⁺, calcd. 387.23).

5.1.102 *tert*-Butyl 4-(benzo[*d*]oxazol-2-yl)piperazine-1-carboxylate (**103**).

2-Chlorobenzo[*d*]oxazole (153 mg, 1.00 mmol) and *tert*-butyl piperazine-1-carboxylate (373 mg, 2.00 mmol) were suspended in H₂O (2 mL) and stirred for 3 d at rt. The precipitate was dissolved in EtOAc, the aqueous phase was extracted 3 times with EtOAc (50 mL) and the organic extracts were combined and dried over MgSO₄. The solvent was removed under reduced pressure to afford 303 mg (0.99 mmol, quant.) of **103**. TLC: *R*_F = 0.67 (SiO₂, cyclohexane/EtOAc, 1:1); ¹H-NMR (500 MHz, CDCl₃, 300 K): δ = 7.36 (d, ³*J* = 7.9 Hz, 1H), 7.25 (d, ³*J* = 7.8 Hz, 1H), 7.17 (t, ³*J* = 7.7 Hz, 1H), 7.03 (t, ³*J* = 7.7 Hz, 1H), 3.71–3.36 (m, 4H), 3.60–3.51 (m, 4H), 1.48 (s, 9H) ppm; ¹³C-NMR (126 MHz, CDCl₃, 300 K): δ = 162.1, 154.7, 148.9, 143.0, 124.2, 121.1, 116.6, 108.9, 80.5, 45.5, 43.3, 28.5 ppm; MS (ESI pos.): *m/z* (%) = 304.25 (14) ([M+H]⁺, calcd. 304.17), 248.20 (100) ([M_{tr}+2H]⁺, calcd. 248.11), 204.12 (49) ([M-Boc+2H]⁺, calcd. 204.12).

5.1.103 2-(Piperazin-1-yl)benzo[d]oxazole (**104**).

tert-Butyl 4-(benzo[d]oxazol-2-yl)piperazine-1-carboxylate (**103**, 500 mg, 1.65 mmol) was dissolved in CH₂Cl₂ (15 mL), cooled to 0 °C and TFA (2 mL) was added. The mixture was stirred for 3 h at rt and the solvent was removed under reduced pressure. The crude product was dissolved in MeOH, K₂CO₃ (3.6 g, 25.96 mmol) was added and the mixture was stirred for 5 min at 5 °C. The mixture was filtered and the solvent was removed under reduced pressure. Water was added and the aqueous phase was extracted 3-times with EtOAc (50 mL). The organic extracts were combined, dried over MgSO₄, and the solvent was evaporated under reduced pressure to afford 298 mg (1.47 mmol, 89%) of **104**. TLC: *R*_F = 0.17 (SiO₂, CH₂Cl₂/MeOH, 10:1); ¹H-NMR (250 MHz, CDCl₃, 300 K): δ = 7.36 (d, ³*J* = 7.5 Hz, 1H), 7.26 (d, ³*J* = 7.9 Hz, 1H), 7.17 (dt, ³*J* = 7.7, 1.1 Hz, 1H), 7.04 (dt, ³*J* = 7.7, 1.1 Hz, 1H), 3.89–3.65 (m, 4H), 3.17–2.96 (m, 4H) ppm; MS (ESI pos.): *m/z* (%) = 204.27 (100) ([M+H]⁺, calcd. 204.11).

5.1.104 (*S*)-(9*H*-Fluoren-9-yl)methyl (1-(4-(benzo[d]oxazol-2-yl)piperazin-1-yl)-4-cyclohexyl-1-oxobutan-2-yl)carbamate (**105**).

Compound **105** was prepared according to general procedure E using (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-cyclohexylbutanoic acid (**71**, 500 mg, 1.23 mmol) and 2-(piperazin-1-yl)benzo[d]oxazole (**104**, 298 mg, 1.47 mmol). The crude product was purified by column chromatography on silica (CH₂Cl₂/MeOH, 10:1) to afford 571 mg (0.96 mmol, 79%) of **105**. TLC: *R*_F = 0.94 (SiO₂, CH₂Cl₂/MeOH, 10:1); ¹H-NMR (400 MHz, DMSO-*d*₆, 300 K): δ = 7.91–7.84 (m, 2H), 7.79–7.69 (m, 2H), 7.63 (d, ³*J* = 8.2 Hz, 1H), 7.45–7.36 (m, 3H), 7.35–7.27 (m, 3H), 7.17 (t, ³*J* = 7.5 Hz, 1H), 7.04 (t, ³*J* = 7.5 Hz, 1H), 4.48–4.39 (m, 1H), 4.33–4.18 (m, 3H), 3.76–3.40 (m, 8H), 1.71–1.50 (m, 7H), 1.27–1.04 (m, 6H), 0.92–0.77 (m, 2H) ppm; ¹³C-NMR (101 MHz, DMSO-*d*₆, 300 K): δ = 170.5, 161.6, 155.9, 148.3, 143.8 (2x), 142.8, 140.7, 127.6, 127.0, 125.3, 124.0, 120.7, 116.0, 109.0, 65.6, 50.8, 46.7, 37.0, 32.9, 32.7, 28.9, 26.1, 25.8, 25.7 ppm; MS (ESI pos.): *m/z* (%) = 371.09 (100) ([M-Fmoc+2H]⁺, calcd. 371.25).

5.1.105 (*S*)-5-Amino-*N*-(4-((4-cyclohexyl-1-(methylamino)-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (136**).**

Compound **136** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 100 mg, 0.29 mmol) and (*S*)-2-amino-4-cyclohexyl-*N*-methylbutanamide (**106**, 71 mg, 0.36 mmol). The crude product was purified by column chromatography on silica (CH₂Cl₂/MeOH 20:1 → 10:1 → EtOH) to afford 90 mg (0.17 mmol, 59%) of **136**. TLC: *R*_F = 0.38 (SiO₂, EtOAc/MeOH 10:1); HPLC: 13.9 min; ¹H-NMR (500 MHz, DMSO-*d*₆, 300 K): δ = 8.51 (t, ³*J* = 6.0 Hz, 1H), 8.33 (d, ³*J* = 8.1 Hz, 1H), 7.96 (s, 1H), 7.89–7.84 (m, 3H), 7.60–7.49 (m, 4H), 7.42–7.33 (m, 3H), 6.38 (s, 2H), 4.47 (d, ³*J* = 6.0 Hz, 2H), 4.38–4.30 (m, 1H), 2.58 (d, ³*J* = 4.6 Hz, 3H), 1.81–1.72 (m, 1H), 1.71–1.54 (m, 6H), 1.28–1.03 (m, 6H), 0.90–0.76 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆, 300 K): δ = 172.3, 166.1, 164.1, 149.2, 143.6, 138.4, 138.2, 132.6, 129.4, 127.6, 127.1, 126.7, 123.1, 97.4, 53.6, 41.3, 36.8, 33.4, 32.9, 32.7, 29.2, 26.2, 25.8 (2x), 25.6 ppm; MS (ESI pos.): *m/z* (%) = 517.20 (100) ([*M*+*H*]⁺, calcd. 517.29), 486.17 (87) ([*M*_{fr.}]⁺, calcd. 486.25), 319.09 (56) ([*M*_{fr. II}]⁺, calcd. 319.25); HRMS (FTMS + p MALDI): *m/z* = 539.2742 [*M*+*Na*]⁺, calcd. for [C₂₉H₃₆N₆NaO₃]⁺ = 539.2747.

5.1.106 (*S*)-5-Amino-*N*-(4-((4-cyclohexyl-1-(ethylamino)-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (137**).**

Compound **137** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 100 mg, 0.30 mmol) and (*S*)-2-amino-4-cyclohexyl-*N*-ethylbutanamide (**107**, 76 mg, 0.36 mmol). The crude product was purified by column chromatography on silica (CH₂Cl₂/MeOH 20:1 → 10:1 → EtOH) to afford 82 mg (0.16 mmol, 52%) of **137**. TLC: *R*_F = 0.25 (SiO₂, CH₂Cl₂/MeOH 20:1); HPLC: 14.4 min; ¹H-NMR (500 MHz, CDCl₃, 300 K): δ = 7.75 (s, 1H), 7.68 (d, ³*J* = 8.2 Hz, 2H), 7.57–7.47 (m, 4H), 7.42–7.36 (m, 1H), 7.29 (d, ³*J* = 8.2 Hz, 2H), 7.08 (d, ³*J* = 8.0 Hz, 1H), 6.67–6.55 (m, 2H), 5.55 (br s, 2H), 4.63–4.51 (m, 3H), 3.36–3.17 (m, 2H), 1.98–1.88 (m, 1H), 1.81–1.57 (m, 6H), 1.33–1.05 (m, 9H), 0.92–0.78 (m, 2H) ppm; ¹³C-NMR (126 MHz, CDCl₃, 300 K): δ = 171.8, 167.2, 164.8, 148.9, 142.9, **137.8**, **137.7**, 132.9, 129.9, 128.2, 127.6, 123.9, 97.9, 54.1, 42.6, 37.7,

34.6, 33.4, 33.3, 30.3 (2x), 26.7, 26.4, 14.8 ppm; MS (ESI pos.): m/z (%) = 531.23 (100) ($[M+H]^+$, calcd. 531.31); HRMS (FTMS + p MALDI): m/z = 531.3063 $[M+H]^+$, calcd. for $[C_{30}H_{39}N_6O_3]^+ = 531.3078$.

5.1.107 (*S*)-5-Amino-*N*-(4-((4-cyclohexyl-1-oxo-1-(propylamino)butan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (138).

Compound **138** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 100 mg, 0.30 mmol) and (*S*)-2-amino-4-cyclohexyl-*N*-propylbutanamide (**108**, 81 mg, 0.36 mmol). The crude product was purified by column chromatography on silica ($CH_2Cl_2/MeOH$ 20:1) to afford 121 mg (0.22 mmol, 75%) of **138**. TLC: R_F = 0.17 (SiO_2 , $CH_2Cl_2/MeOH$ 20:1); HPLC: 14.9 min; 1H -NMR (500 MHz, $DMSO-d_6$, 300 K): δ = 8.55–8.47 (m, 1H), 8.28 (d, 3J = 8.1 Hz, 1H), 7.98 (s, 1H), 7.92 (t, 3J = 5.7 Hz, 1H), 7.85 (d, 3J = 8.3 Hz, 2H), 7.59–7.49 (m, 4H), 7.41–7.31 (m, 3H), 6.38 (br s, 2H), 4.49–4.43 (m, 2H), 4.39–4.32 (m, 1H), 3.10–2.93 (m, 2H), 1.81–1.71 (m, 1H), 1.70–1.55 (m, 6H), 1.40 (sext, 3J = 7.3 Hz, 2H), 1.28–1.04 (m, 6H), 0.90–0.77 (m, 5H) ppm; ^{13}C -NMR (126 MHz, $DMSO-d_6$, 300 K): δ = 171.7, 168.8, 166.0, 164.1, 149.2, 143.6, 141.4, 138.4, 138.2, 134.9, 132.7, 129.4, 127.6, 127.1, 127.0, 126.8, 126.7, 123.1, 97.4, 53.6, 41.3, 36.8, 33.3, 32.9, 32.7, 29.4, 26.2, 25.8 (2x), 24.1, 22.3, 11.4 ppm; MS (ESI pos.): m/z (%) = 545.32 (100) ($[M+H]^+$, calcd. 545.33), 486.27 (30) ($[M_{fr.}]^+$, calcd. 486.25); HRMS (FTMS + p MALDI): m/z = 545.3222 $[M+H]^+$, calcd. for $[C_{31}H_{41}N_6O_3]^+ = 545.3240$.

5.1.108 (*S*)-5-Amino-*N*-(4-((1-(butylamino)-4-cyclohexyl-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (139).

Compound **139** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 100 mg, 0.30 mmol) and (*S*)-2-amino-4-cyclohexyl-*N*-butylbutanamide (**109**, 120 mg, 0.50 mmol). The crude product was purified by column chromatography on silica ($CH_2Cl_2/MeOH$ 20:1) to afford 140 mg (0.25 mmol, 85%) of **139**. TLC: R_F = 0.30 (SiO_2 , $CH_2Cl_2/MeOH$ 20:1); HPLC: 15.5 min; 1H -NMR (500 MHz, $DMSO-d_6$, 300 K): δ = 8.51 (t, 3J = 6.1 Hz, 1H), 8.27 (d, 3J = 8.0 Hz, 1H), 7.98 (s, 1H), 7.91 (t, 3J = 5.1 Hz, 1H), 7.85 (d, 3J = 8.1 Hz, 2H), 7.61–

7.48 (m, 4H), 7.42–7.35 (m, 3H), 6.38 (br s, 2H), 4.47 (d, $^3J = 6.0$ Hz, 2H), 4.39–4.31 (m, 1H), 3.14–2.97 (m, 2H), 1.80–1.71 (m, 1H), 1.70–1.55 (m, 6H), 1.42–1.33 (m, 2H), 1.31–1.04 (m, 8H), 0.91–0.77 (m, 5H) ppm; ^{13}C -NMR (126 MHz, DMSO- d_6 , 300 K): $\delta = 171.6, 166.0, 164.1, 149.2, 143.6, 138.4, 138.2, 132.7, 129.4, 127.6, 126.8, 123.1, 97.4, 53.6, 41.3, 38.1, 36.8, 33.3, 32.9, 32.7, 31.2, 29.4, 26.2, 25.8$ (2x), 19.5, 13.7 ppm; MS (ESI neg.): m/z (%) = 557.47 (100) ($[\text{M}-\text{H}]^-$, calcd. 557.31), 603.44 (41) ($[\text{M}+\text{EtOH}-\text{H}]^-$, calcd. 603.36); HRMS (FTMS + p MALDI): $m/z = 581.3211$ $[\text{M}+\text{Na}]^+$, calcd. for $[\text{C}_{32}\text{H}_{42}\text{N}_6\text{NaO}_3]^+ = 581.3216$.

5.1.109 (*S*)-5-Amino-*N*-(4-((4-cyclohexyl-1-(hexylamino)-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (140).

Compound **140** was prepared according to general procedure B using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 150 mg, 0.45 mmol) and (*S*)-2-amino-4-cyclohexyl-*N*-hexylbutanamide (**110**, 144 mg, 0.54 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 1:1 $\rightarrow$ 1:6 $\rightarrow$ EtOAc) to afford 206 mg (0.35 mmol, 79%) of **140**. TLC: $R_F = 0.33$ (SiO_2 , cyclohexane/EtOAc 1:6); HPLC: 16.7 min; ^1H -NMR (500 MHz, DMSO- d_6 , 300 K): $\delta = 8.52$ (t, $^3J = 6.1$ Hz, 1H), 8.28 (d, $^3J = 8.1$ Hz, 1H), 7.99 (s, 1H), 7.92 (t, $^3J = 5.6$ Hz, 1H), 7.86 (d, $^3J = 8.3$ Hz, 2H), 7.60–7.49 (m, 4H), 7.41–7.35 (m, 3H), 6.39 (br s, 2H), 4.47 (d, $^3J = 5.9$ Hz, 2H), 4.39–4.31 (m, 1H), 3.16–3.06 (m, 1H), 3.04–2.94 (m, 1H), 1.81–1.55 (m, 7H), 1.43–1.32 (m, 2H), 1.31–1.04 (m, 12H), 0.91–0.77 (m, 5H) ppm; ^{13}C -NMR (126 MHz, DMSO- d_6 , 300 K): $\delta = 171.6, 166.0, 164.1, 149.2, 143.6, 138.4, 138.2, 132.7, 129.4, 127.6, 127.1, 126.8, 123.1, 97.4, 53.6, 41.3, 38.4, 36.8, 33.3, 32.9, 32.7, 31.0, 29.4, 29.0, 26.2, 26.0, 25.8, 22.1, 13.9$ ppm; MS (ESI pos.): m/z (%) = 587.38 (48) ($[\text{M}+\text{H}]^+$, calcd. 587.37), 486.27 (13) ($[\text{M}_{\text{fr.I}}]^+$, calcd. 486.25), 190.35 (100) ($[\text{M}_{\text{fr.II}}+\text{Na}]^+$, calcd. 190.13); HRMS (FTMS + p MALDI): $m/z = 609.3523$ $[\text{M}+\text{Na}]^+$, calcd. for $[\text{C}_{34}\text{H}_{46}\text{N}_6\text{NaO}_3]^+ = 609.3529$.

5.1.110 (*S*)-5-Amino-*N*-(4-((4-cyclohexyl-1-(decylamino)-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (141).

Compound **141** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-2-amino-4-cyclohexyl-*N*-decylbutanamide (**111**, 239 mg, 0.74 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 1:6 → EtOAc) to afford 217 mg (0.34 mmol, 57%) of **141**. TLC: R_F = 0.41 (SiO₂, cyclohexane/EtOAc 1:6); HPLC: 21.0 min; ¹H-NMR (500 MHz, DMSO-*d*₆, 300 K): δ = 8.51 (t, ³*J* = 5.9 Hz, 1H), 8.27 (d, ³*J* = 8.0 Hz, 1H), 7.98 (s, 1H), 7.91 (t, ³*J* = 5.6 Hz, 1H), 7.85 (d, ³*J* = 8.3 Hz, 2H), 7.59–7.50 (m, 4H), 7.41–7.35 (m, 3H), 6.38 (br s, 2H), 4.47 (d, ³*J* = 5.9 Hz, 2H), 4.39–4.31 (m, 1H), 3.16–3.06 (m, 1H), 3.03–2.94 (m, 1H), 1.80–1.71 (m, 1H), 1.70–1.55 (m, 6H), 1.44–1.33 (m, 2H), 1.32–1.03 (m, 20H), 0.90–0.77 (m, 5H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆, 300 K): δ = 171.6, 166.0, 164.2, 149.2, 143.6, 138.4, 138.2, 132.7, 129.4, 127.6, 127.1, 126.8, 123.1, 97.4, 53.7, 41.3, 36.9, 33.3, 32.9, 32.8, 31.3, 29.4, 29.1, 29.0 (2x), 28.8, 26.3, 26.2, 25.8, 22.1, 14.0 ppm; MS (ESI pos.): *m/z* (%) = 643.46 (96) ([*M*+*H*]⁺, calcd. 643.44), 486.33 (25) ([*M*_{fr.I}]⁺, calcd. 486.25), 190.35 (100) ([*M*_{fr.II}+*Na*]⁺, calcd. 190.13); HRMS (FTMS + p MALDI): *m/z* = 665.4169 [*M*+*Na*]⁺, calcd. for [C₃₈H₅₄N₆NaO₃]⁺ = 466.4155.

5.1.111 5-Amino-*N*-(4-(((*S*)-1-((*SR*)-*sec*-butylamino)-4-cyclohexyl-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (**142**).

Compound **142** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 150 mg, 0.446 mmol) and (*S*)-2-amino-*N*-((*SR*)-*sec*-butyl)-4-cyclohexylbutanamide (**112**, 81 mg, 0.36 mmol). The crude product was purified by column chromatography on silica (CH₂Cl₂/MeOH 20:1) to afford 155 mg (0.28 mmol, 78%) of **142**. TLC: R_F = 0.19 (SiO₂, CH₂Cl₂/MeOH 20:1); HPLC: 15.5 min; ¹H-NMR (500 MHz, DMSO-*d*₆, 300 K): δ = 8.51 (t, ³*J* = 5.9 Hz, 1H), 8.22 (dd, ³*J* = 8.0, 12.3 Hz, 1H), 7.98 (s, 1H), 7.85 (d, ³*J* = 8.0 Hz, 2H), 7.72 (dd, ³*J* = 8.2, 20.7 Hz, 1H), 7.59–7.49 (m, 4H), 7.41–7.34 (m, 3H), 6.38 (br s, 2H), 4.47 (d, ³*J* = 5.8 Hz, 2H), 4.40–4.32 (m, 1H), 3.67 (sext, ³*J* = 6.7 Hz, 1H), 1.78–1.55 (m, 7H), 1.43–1.32 (m, 2H), 1.28–1.08 (m, 6H), 1.01 (dd, ³*J* = 6.7, 10.3 Hz, 3H), 0.88–0.77 (m, 5H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆, 300 K): δ = 171.7, 165.9 (2x), 164.2, 149.2, 143.6, 138.4, 138.2, 132.7, 129.4, 127.5, 127.1, 126.8, 123.1, 97.4, 53.6 (2x), 45.7 (2x),

41.3, 36.8, 33.3, 33.0, 32.7, 29.4, 28.8 (2x), 26.2, 25.8 (2x), 20.3 (2x), 10.5, 10.4 ppm; MS (ESI pos.): m/z (%) = 581.39 (100) ($[M+Na]^+$, calcd. 581.32); HRMS (FTMS + p MALDI): m/z = 581.3209 $[M+Na]^+$, calcd. for $[C_{32}H_{42}N_6NaO_3]^+$ = 581.3216.

5.1.112 (*S*)-5-Amino-*N*-(4-((4-cyclohexyl-1-oxo-1-(pentan-3-ylamino)butan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (143).

Compound **143** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 150 mg, 0.45 mmol) and (*S*)-2-amino-4-cyclohexyl-*N*-(pentan-3-yl)butanamide (**113**, 136 mg, 0.54 mmol). The crude product was purified by column chromatography on silica ($CH_2Cl_2/MeOH$ 20:1) to afford 148 mg (0.26 mmol, 58%) of **143**. TLC: R_F = 0.20 (SiO_2 , $CH_2Cl_2/MeOH$ 20:1); HPLC: 15.9 min; 1H -NMR (500 MHz, $CDCl_3$, 300 K): δ = 7.76 (s, 1H), 7.67 (d, 3J = 8.2 Hz, 2H), 7.56–7.46 (m, 4H), 7.38 (t, 3J = 7.2 Hz, 1H), 7.29 (d, 3J = 8.2 Hz, 2H), 7.15 (d, 3J = 7.9 Hz, 1H), 6.66 (t, 3J = 5.9 Hz, 1H), 6.33 (d, 3J = 9.0 Hz, 1H), 5.52 (s, 2H), 4.65–4.51 (m, 3H), 3.78–3.68 (m, 1H), 2.00–1.88 (m, 1H), 1.82–1.72 (m, 1H), 1.71–1.59 (m, 5H), 1.58–1.44 (m, 2H), 1.43–1.05 (m, 8H), 0.93–0.78 (m, 8H) ppm; ^{13}C -NMR (126 MHz, $DMSO-d_6$, 300 K): δ = 171.7, 167.1, 164.8, 149.0, 142.9, **137.8**, **137.7**, 133.0, 129.9, 128.2, 127.6 (2x), 123.9, 98.0, 54.3, 52.5, 42.6, 37.7, 33.4, 33.3, 33.2, 30.4, 27.5 (2x), 26.7, 26.4, 10.5, 10.4 ppm; MS (ESI pos.): m/z (%) = 573.27 (100) ($[M+H]^+$, calcd. 573.36), 486.20 (61) ($[M_{fr.}]^+$, calcd. 486.25); HRMS (FTMS + p MALDI): m/z = 573.3540 $[M+H]^+$, calcd. for $[C_{33}H_{45}N_6O_3]^+$ = 573.3553.

5.1.113 5-Amino-*N*-(4-(((*S*)-4-cyclohexyl-1-(((*SR*)-1-hydroxybutan-2-yl)amino)-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (144).

Compound **144** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-2-amino-4-cyclohexyl-*N*-((*SR*)-1-hydroxybutan-2-yl)butanamide (**114**, 189 mg, 0.71 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 1:20) to afford 166 mg (0.29 mmol, 49%) of **144**. TLC: R_F = 0.76 (SiO_2 , EtOAc/MeOH 10:1); HPLC: 13.78, 14.1 min; 1H -NMR (500 MHz, $DMSO-d_6$, 300 K):

(mixture of diastereomers) δ = 8.52 (t, 3J = 6.1 Hz, 1H), 8.30 (d, 3J = 8.1 Hz, 0.7H), 8.25 (d, 3J = 8.1 Hz, 0.3H), 7.99 (s, 1H), 7.85 (d, 3J = 8.3 Hz, 2H), 7.68 (d, 3J = 8.5 Hz, 0.3H), 7.60–7.49 (m, 4.7H), 7.42–7.33 (m, 3H), 6.38 (br s, 2H), 4.65–4.58 (m, 1H), 4.47 (d, 3J = 5.9 Hz, 2H), 4.45–4.34 (m, 1H), 3.66–3.56 (m, 1H), 3.40–3.30 (m, 1H), 3.29–3.20 (m, 1H), 1.81–1.53 (m, 7H), 1.36–1.04 (m, 7H), 0.90–0.77 (m, 5H) ppm; ^{13}C -NMR (126 MHz, DMSO- d_6 , 300 K): (mixture of diastereomers) δ = 171.7, 166.0 (2x), 164.2, 149.2, 143.9, 138.4, 138.2, 132.7, 129.4, 127.5 (2x), 127.1, 126.8, 123.1, 97.4, 63.0 (2x), 53.8, 53.7, 52.2, (2x), 41.4, 36.9, 36.8, 33.3, 33.2, 33.0, 32.7, 29.7, 29.4, 26.2, 25.8 (2x), 23.7, 10.4 (2x) ppm; MS (ESI pos.): m/z (%) = 575.17 (100) ($[\text{M}+\text{H}]^+$, calcd. 575.34), 486.08 (33) ($[\text{M}_{\text{fr.I}}]^+$, calcd. 486.25), 319.04 (12) ($[\text{M}_{\text{fr.II}}]^+$, calcd. 319.12); HRMS (FTMS + p MALDI): m/z = 597.3173 $[\text{M}+\text{Na}]^+$, calcd. for $[\text{C}_{32}\text{H}_{42}\text{N}_6\text{NaO}_4]^+$ = 597.3165.

5.1.114 (*S*)-5-Amino-*N*-(4-((4-cyclohexyl-1-(isopentylamino)-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (**145**).

Compound **145** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-2-amino-4-cyclohexyl-*N*-isopentylbutanamide (**115**, 182 mg, 0.71 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 1:1 $\rightarrow$ 1:6 $\rightarrow$ EtOAc) to afford 327 mg (0.57 mmol, 96%) of **145**. TLC: R_F = 0.41 (SiO₂, cyclohexane/EtOAc 1:6); HPLC: 16.0 min; ^1H -NMR (500 MHz, DMSO- d_6 , 300 K): δ = 8.52 (t, 3J = 6.1 Hz, 1H), 8.27 (d, 3J = 8.0 Hz, 1H), 7.99 (s, 1H), 7.90 (t, 3J = 5.6 Hz, 1H), 7.85 (d, 3J = 8.3 Hz, 2H), 7.59–7.49 (m, 4H), 7.41–7.35 (m, 3H), 6.38 (br s, 2H), 4.47 (d, 3J = 6.0 Hz, 2H), 4.38–4.31 (m, 1H), 3.16–2.99 (m, 2H), 1.80–1.71 (m, 1H), 1.70–1.52 (m, 6H), 1.33–1.03 (m, 9H), 0.90–0.79 (m, 8H) ppm; ^{13}C -NMR (126 MHz, DMSO- d_6 , 300 K): δ = 171.5, 166.0, 164.1, 149.2, 143.6, 138.4, 138.2, 132.7, 129.4, 127.6, 127.1, 126.8, 123.1, 97.4, 53.6, 41.3, 38.1, 36.8, 36.7, 33.3, 32.9, 32.7, 29.3, 26.2, 25.8, 25.1, 22.4 ppm; MS (ESI pos.): m/z (%) = 573.38 (100) ($[\text{M}+\text{H}]^+$, calcd. 573.35), 486.30 (26) ($[\text{M}_{\text{fr.I}}]^+$, calcd. 486.25), 319.22 (22) ($[\text{M}_{\text{fr.II}}]^+$, calcd. 319.12); HRMS (FTMS + p MALDI): m/z = 573.3530 $[\text{M}+\text{H}]^+$, calcd. for $[\text{C}_{33}\text{H}_{45}\text{N}_6\text{O}_3]^+$ = 573.3548.

5.1.115 (*S*)-5-Amino-*N*-(4-((4-cyclohexyl-1-(cyclohexylamino)-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (146**).**

Compound **146** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-2-amino-*N*,4-dicyclohexylbutanamide (**116**, 159 mg, 0.71 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 2:3 → 1:6 → EtOAc) to afford 138 mg (0.24 mmol, 40%) of **146**. TLC: R_F = 0.38 (SiO₂, cyclohexane/EtOAc 1:6); HPLC: 16.3 min; ¹H-NMR (500 MHz, DMSO-*d*₆, 300 K): δ = 8.52 (t, ³*J* = 6.0 Hz, 1H), 8.24 (d, ³*J* = 8.2 Hz, 1H), 7.99 (s, 1H), 7.89–7.78 (m, 3H), 7.60–7.49 (m, 4H), 7.42–7.30 (m, 3H), 6.39 (br s, 2H), 4.48 (d, ³*J* = 6.0 Hz, 2H), 4.42–4.33 (m, 1H), 3.61–3.47 (m, 1H), 1.78–1.48 (m, 12H), 1.33–1.03 (m, 11H), 0.90–0.76 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆, 300 K): δ = 170.8, 165.9, 164.1, 149.2, 143.6, 138.4, 138.2, 132.7, 129.4, 127.5, 127.1, 126.8, 123.1, 97.4, 59.8, 53.5, 47.5, 41.3, 36.8, 33.3, 32.9, 32.7, 32.4, 32.2, 29.6, 29.2, 26.2, 25.8, 25.2, 24.5 ppm; MS (ESI pos.): *m/z* (%) = 585.25 (100) ([*M*+H]⁺, calcd. 585.36), 486.16 (80) ([*M*_{fr}]⁺, calcd. 486.25); HRMS (FTMS + p MALDI): *m/z* 607.3360 [*M*+Na]⁺, calcd. for [C₃₄H₄₄N₆NaO₃]⁺ = 607.3376.

5.1.116 (*S*)-5-Amino-*N*-(4-((4-cyclohexyl-1-morpholino-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (147**).**

Compound **147** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-2-amino-4-cyclohexyl-1-morpholinobutan-1-one (**117**, 182 mg, 0.71 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 1:3 → 1:10) to afford 90 mg (0.16 mmol, 26%) of **147**. TLC: R_F = 0.27 (cyclohexane/EtOAc 1:10); HPLC: 14.6 min; ¹H-NMR (500 MHz, DMSO-*d*₆, 300 K): δ = 8.54 (d, ³*J* = 8.1 Hz, 1H), 8.52 (t, ³*J* = 6.1 Hz, 1H), 7.99 (s, 1H), 7.86 (d, ³*J* = 8.3 Hz, 2H), 7.59–7.55 (m, 2H), 7.52 (t, ³*J* = 7.9 Hz, 2H), 7.41–7.35 (m, 3H), 6.38 (br s, 2H), 4.86–4.78 (m, 1H), 4.47 (d, ³*J* = 5.9 Hz, 2H), 3.61–3.39 (m, 8H), 1.77–1.55 (m, 7H), 1.27–1.05 (m, 6H), 0.90–0.78 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆, 300 K): δ = 170.1, 165.8, 164.2, 149.2, 143.7, 138.4, 138.2, 132.4, 129.4, 127.6, 127.1, 126.8,

123.1, 97.4, 66.2, 49.3, 45.6, 42.0, 41.3, 37.0, 33.1, 32.9, 32.7, 28.8, 26.2, 25.8 (2x) ppm; MS (ESI pos.): m/z (%) = 573.33 (51) ($[M+H]^+$, calcd. 573.32), 486.28 (23) ($[M_{fr.1}]^+$, calcd. 486.25), 190.35 (100) ($[M_{fr.11}+Na]^+$, calcd. 190.13); HRMS(FTMS + p MALDI): m/z = 595.2997 $[M+Na]^+$, calcd. for $[C_{32}H_{40}N_6NaO_4]^+$ = 595.3009.

5.1.117 (S)-5-Amino-N-(4-((4-cyclohexyl-1-oxo-1-(piperazin-1-yl)butan-2-yl)carbamoyl)benzyl)-1-phenyl-1H-pyrazole-4-carboxamide (148).

(S)-tert-Butyl-4-(2-(4-((5-amino-1-phenyl-1H-pyrazole-4-carboxamido)methyl)benzamido)-4-cyclohexylbutanoyl)piperazine-1-carboxylate (**162**, 80 mg, 0.120 mmol, 1 eq) was dissolved in CH_2Cl_2 (10 mL) and cooled to 0 °C. TFA (2 mL) was added dropwise, and the mixture was stirred for 1.5 h at rt. The solvent was removed under reduced pressure, and the crude product was dissolved in MeOH. The solution was cooled to 5 °C and neutralized with K_2CO_3 . After 10 min stirring, the mixture was filtered, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 1:9 → EtOAc → EtOH+1% TEA) to afford 35 mg (0.06 mmol, 51%) of **148**. TLC: R_F = 0.04 (EtOAc/MeOH 10:1); HPLC: 10.1 min; 1H -NMR (500 MHz, DMSO- d_6 , 300 K): δ = 8.56 (t, 3J = 8.2 Hz, 1H), 8.50 (t, 3J = 8.2 Hz 2H), 8.00 (s, 1H), 7.84 (d, 3J = 8.2 Hz, 2H), 7.60–7.49 (m, 4H), 7.41–7.34 (m, 3H), 6.38 (br s, 2H), 4.88–4.76 (m, 1H), 4.46 (d, 3J = 5.9 Hz, 2H), 3.64–3.45 (m, 4H), 2.47–2.29 (m, 4H), 1.76–1.54 (m, 7H), 1.29–1.04 (m, 7H), 0.90–0.77 (m, 2H) ppm; ^{13}C -NMR (126 MHz, DMSO- d_6 , 300 K): δ = 169.8, 165.8, 164.2, 149.2, 143.7, 138.4, 138.2, 132.4, 129.4, 127.6, 127.1, 126.8, 123.1, 97.4, 51.5, 50.9, 45.1, 41.5, 41.3, 37.0, 33.1, 32.9, 32.7, 29.0, 26.2, 25.8 ppm; MS (ESI pos.): m/z (%) = 572.43 (100) ($[M+H]^+$, calcd. 572.34); HRMS (FTMS + p MALDI): m/z = 572.3328 $[M+H]^+$, calcd. for $[C_{32}H_{42}N_7O_3]^+$ = 572.3344.

5.1.118 (S)-tert-Butyl-4-(2-(4-((5-amino-1-phenyl-1H-pyrazole-4-carboxamido)methyl)benzamido)-4-cyclohexylbutanoyl)piperazine-1-carboxylate (149).

Compound **149** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-*tert*-butyl 4-(2-amino-4-cyclohexylbutanoyl)piperazine-1-carboxylate (**131**, 252 mg, 0.71 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc) to afford 138 mg (0.21 mmol, 35%) of **149**. TLC: R_F = 0.58 (EtOAc/MeOH 10:1); HPLC: 16.4 min; $^1\text{H-NMR}$ (500 MHz, DMSO- d_6 , 300 K): δ = 8.58–8.47 (m, 2H), 7.98 (s, 1H), 7.85 (d, 3J = 8.2 Hz, 2H), 7.59–7.49 (m, 4H), 7.41–7.32 (m, 3H), 6.38 (br s, 2H), 4.87–4.79 (m, 1H), 4.47 (d, 3J = 5.9 Hz, 2H), 3.64–3.18 (m, 8H), 1.77–1.54 (m, 7H), 1.40 (s, 9H), 1.28–1.05 (m, 6H), 0.91–0.78 (m, 2H) ppm; $^{13}\text{C-NMR}$ (126 MHz, DMSO- d_6 , 300 K): δ = 170.2, 165.9, 164.1, 159.3, 149.2, 143.7, 138.4, 138.2, 132.4, 129.4, 127.6, 127.1, 126.8, 123.1, 97.4, 79.2, 59.8, 49.5, 44.7, 41.4, 41.3, 38.2, 37.0, 33.1, 32.9, 32.7, 28.8, 26.2, 25.8 ppm; MS (ESI pos.): m/z (%) = 672.42 (24) ($[\text{M-H}]^+$, calcd. 672.42), 486.26 (11) ($[\text{M}_{\text{fr.1}}]^+$, calcd. 486.26), 190.36 (100) ($[\text{M}_{\text{fr.11}}+\text{Na}]^+$, calcd. 6190.13); HRMS (FTMS + p MALDI): m/z = 694.3688 $[\text{M}+\text{Na}]^+$, calcd. for $[\text{C}_{37}\text{H}_{49}\text{N}_7\text{NaO}_5]^+$ = 694.3693.

5.1.119 (*S*)-5-Amino-*N*-(4-((4-cyclohexyl-1-((4-fluorophenyl)amino)-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (**150**).

Compound **150** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-2-amino-4-cyclohexyl-*N*-(4-fluorophenyl)butanamide (**118**, 270 mg, 0.71 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 1:1 → 1:3 → 1:20) and further recrystallized from acetone to afford 149 mg (0.25 mmol, 42%) of **150**. TLC: R_F = 0.73 (SiO_2 , cyclohexane/EtOAc 1:3); HPLC: 16.1 min; $^1\text{H-NMR}$ (600 MHz, DMSO- d_6 , 300 K): δ = 10.15 (s, 1H), 8.57–8.44 (m, 2H), 7.99 (s, 1H), 7.89 (d, 3J = 7.5 Hz, 2H), 7.68–7.48 (m, 6H), 7.43–7.35 (m, 3H), 7.14 (t, 3J = 8.1 Hz, 2H), 6.37 (br s, 2H), 4.56–4.43 (m, 3H), 1.88–1.75 (m, 2H), 1.73–1.56 (m, 5H), 1.39–1.30 (m, 1H), 1.28–1.05 (m, 5H), 0.92–0.81 (m, 2H) ppm; $^{13}\text{C-NMR}$ (150 MHz, DMSO- d_6 , 300 K): δ = 170.9, 166.3, 164.1, 157.9 ($^1J_{\text{CF}}$ = 240.8 Hz), 149.2, 143.6, 138.3, 138.2, 135.3, 132.4, 129.3, 127.6, 127.0, 126.7, 123.0, 121.0 ($^3J_{\text{CF}}$ = 7.9 Hz), 115.2 ($^2J_{\text{CF}}$ = 22.3 Hz), 97.4, 54.5, 41.3, 36.8, 33.3, 32.8, 32.6, 29.1, 26.1, 25.8 (2x) ppm; MS (ESI pos.): m/z (%) =

619.20 (13) ($[M+Na]^+$, calcd. 619.28), 597.20 (100) ($[M+H]^+$, calcd. 597.31), 486.19 (99) ($[M_{fr.I}]^+$, calcd. 486.25), 458.21 (16) ($[M_{fr.II}]^+$, calcd. 458.26), 319.04 (28) ($[M_{fr.III}]^+$, calcd. 319.12); HRMS (FTMS + p MALDI): m/z = 619.2816 $[M+Na]^+$, calcd. for $[C_{34}H_{37}FN_6NaO_3]^+ = 619.2809$.

5.1.120 5-Amino-*N*-(4-((1-((4-chlorophenyl)amino)-4-cyclohexyl-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (151).

Compound **151** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 185 mg, 0.55 mmol) and (*S*)-2-amino-*N*-(4-chlorophenyl)-4-cyclohexyl-butanamide (**119**, 195 mg, 0.66 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 1:1 $\rightarrow$ 1:3 $\rightarrow$ 1:20) to afford 91 mg (0.148 mmol, 27%) of **151**. TLC: R_F = 0.40 (SiO₂, cyclohexane/EtOAc 1:3); HPLC: 16.8 min; ¹H-NMR (500 MHz, DMSO-*d*₆, 300 K): δ = 10.26 (s, 1H), 8.60–8.48 (m, 2H), 7.99 (s, 1H), 7.88 (d, ³*J* = 8.0 Hz, 2H), 7.65 (d, ³*J* = 8.6 Hz, 2H), 7.60–7.49 (m, 4H), 7.44–7.31 (m, 5H), 6.38 (br s, 2H), 4.56–4.42 (m, 3H), 1.88–1.75 (m, 2H), 1.73–1.55 (m, 5H), 1.40–1.29 (m, 1H), 1.28–1.04 (m, 5H), 0.92–0.79 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆, 300 K): δ = 172.3, 166.4, 164.2, 149.2, 143.7, 138.4, 138.2, 138.0, 132.4, 129.4, 128.6, 127.7, 127.1, 126.8, 123.1, 120.8, 97.4, 54.7, 41.3, 36.9, 33.4, 32.9, 32.7, 29.1, 26.1, 25.8 (2x) ppm; MS (ESI pos.): m/z (%) = 613.21 (76) ($[M+H]^+$, calcd. 613.27), 486.20 (100) ($[M_{fr.}]^+$, calcd. 486.26); HRMS (FTMS + p MALDI): m/z = 613.2684 $[M+H]^+$, calcd. for $[C_{34}H_{38}ClN_6O_3]^+ = 613.2694$.

5.1.121 (*S*)-5-Amino-*N*-(4-((1-((4-bromophenyl)amino)-4-cyclohexyl-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (152).

Compound **152** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-2-amino-*N*-(4-bromophenyl)-4-cyclohexyl-butanamide (**120**, 204 mg, 0.60 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 1:1 $\rightarrow$ 1:3 $\rightarrow$ 1:20) and further recrystallized from acetone to afford 69 mg (0.10 mmol, 18%) of **152**. TLC: R_F = 0.17 (SiO₂, cyclohexane/EtOAc 1:1); HPLC: 17.0 min; ¹H-NMR (600 MHz, DMSO-*d*₆, 300 K): δ = 10.23 (s, 1H), 8.55–8.48 (m, 2H), 7.98 (s, 1H), 7.88 (d, ³*J* = 8.2

Hz, 2H), 7.60 (d, $^3J = 9.0$ Hz, 2H), 7.57 (d, $^3J = 7.8$ Hz, 2H), 7.52 (t, $^3J = 8.0$ Hz, 2H), 7.48 (d, $^3J = 9.0$ Hz, 2H), 7.41–7.37 (m, 3H), 6.37 (br s, 2H), 4.54–4.46 (m, 3H), 1.87–1.75 (m, 2H), 1.72–1.55 (m, 5H), 1.37–1.29 (m, 1H), 1.28–1.06 (m, 5H), 0.92–0.81 (m, 2H) ppm; ^{13}C -NMR (75 MHz, DMSO- d_6 , 300 K): $\delta = 171.3, 166.4, 164.2, 149.2, 143.7, 138.4, 138.2, 132.4, 131.5, 129.4, 127.6, 127.1, 126.8, 123.1, 121.2, 114.8, 97.4, 54.7, 41.3, 36.9, 33.4, 32.9, 32.7, 29.1, 26.1, 25.8$ (2x) ppm; MS (ESI pos.): m/z (%) = 681.14 (15) ($[\text{M}+\text{Na}]^+$, calcd. 681.20), 659.14 (41) ($[\text{M}+\text{H}]^+$, calcd. 659.22), 486.19 (100) ($[\text{M}_{\text{fr.I}}]^+$, calcd. 486.25), 392.00 (60) ($[\text{M}_{\text{fr.II}}]^+$, calcd. 392.24), 324.21 (57) ($[\text{M}_{\text{fr.III}}]^+$, calcd. 324.10); HRMS (FTMS + p MALDI): $m/z = 679.2019$ $[\text{M}+\text{Na}]^+$, calcd. for $[\text{C}_{34}\text{H}_{37}\text{BrN}_6\text{NaO}_3]^+ = 679.2008$.

5.1.122 (*S*)-5-Amino-*N*-(4-((4-cyclohexyl-1-((3,5-difluorophenyl)amino)-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (153).

Compound **153** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-2-amino-4-cyclohexyl-*N*-(3,5-difluorophenyl)butanamide (**121**, 178 mg, 0.60 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 1:1 $\rightarrow$ 1:3 $\rightarrow$ 1:20) and further recrystallized from acetone to afford 202 mg (0.33 mmol, 55%) of **153**. TLC: $R_F = 0.19$ (SiO₂, cyclohexane/EtOAc 1:1); HPLC: 16.7 min; ^1H -NMR (600 MHz, DMSO- d_6 , 300 K): $\delta = 10.50$ (s, 1H), 8.58 (d, $^3J = 7.4$ Hz, 1H), 8.48 (t, $^3J = 5.9$ Hz, 1H), 8.00 (s, 1H), 7.90 (d, $^3J = 8.2$ Hz, 2H), 7.58 (d, $^3J = 7.9$ Hz, 2H), 7.53 (t, $^3J = 8.0$ Hz, 2H), 7.43–7.34 (m, 5H), 6.91 (tt, $^3J = 9.2, 2.0$ Hz, 1H), 6.38 (br s, 2H), 4.53–4.46 (m, 3H), 1.88–1.77 (m, 2H), 1.74–1.57 (m, 5H), 1.40–1.32 (m, 1H), 1.29–1.07 (m, 5H), 0.92–0.82 (m, 2H) ppm; ^{13}C -NMR (75 MHz, DMSO- d_6 , 300 K): $\delta = 171.9, 166.5, 164.1, 162.5$ ($^1J_{\text{CF}} = 243.3$ Hz), 162.3 ($^1J_{\text{CF}} = 243.3$ Hz), 149.2, 143.8, 141.7 (2x), 141.3, 138.4, 138.2, 132.3, 129.4, 127.6, 127.1, 126.8, 123.1, 102.1 ($^2J_{\text{CF}} = 19.3$ Hz), 102.0 ($^2J_{\text{C-F}} = 19.3$ Hz), 98.7, 98.4, 98.0, 97.4, 54.8, 41.3, 36.9, 33.4, 32.9, 32.7, 28.9, 26.1, 25.8 (2x) ppm; MS (ESI pos.): m/z (%) = 615.26 (100) ($[\text{M}-\text{H}]^+$, calcd. 615.28), 486.11 (69) ($[\text{M}_{\text{fr.}}]^+$, calcd. 486.25); HRMS (FTMS + p MALDI): $m/z = 615.2887$ $[\text{M}+\text{H}]^+$, calcd. for $[\text{C}_{34}\text{H}_{37}\text{F}_2\text{N}_6\text{O}_3]^+ = 615.2895$, 637.2710 $[\text{M}+\text{Na}]^+$, calcd. for $[\text{C}_{34}\text{H}_{36}\text{F}_2\text{N}_6\text{NaO}_3]^+ = 637.2709$.

5.1.123 5-Amino-*N*-(4-((4-cyclohexyl-1-((3,5-dichlorophenyl)amino)-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (154).

Compound **154** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 140 mg, 0.42 mmol) and (*S*)-2-amino-4-cyclohexyl-*N*-(3,5-dichlorophenyl)butanamide (**122**, 165 mg, 0.50 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 1:1 → 1:3 → 1:20 → EtOAc) and further recrystallized from acetone to afford 97 mg (0.15 mmol, 32%) of **154**. TLC: R_F = 0.48 (SiO₂, cyclohexane/EtOAc 1:3); HPLC: 18.27 min; ¹H-NMR (500 MHz, DMSO-*d*₆, 300 K): δ = 10.49 (s, 1H), 8.60 (d, ³*J* = 7.3 Hz, 1H), 8.53 (t, ³*J* = 6.1 Hz, 1H), 7.99 (s, 1H), 7.88 (d, ³*J* = 8.3 Hz, 2H), 7.71 (d, ⁴*J* = 1.9 Hz, 2H), 7.58–7.49 (m, 4H), 7.42–7.36 (m, 3H), 7.28 (t, ⁴*J* = 1.9 Hz, 1H), 6.39 (br s, 2H), 4.51–4.23 (m, 3H), 1.88–1.75 (m, 2H), 1.73–1.55 (m, 5H), 1.39–1.30 (m, 1H), 1.28–1.05 (m, 5H), 0.91–0.80 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆, 300 K): δ = 172.0, 166.6, 164.2, 149.2, 143.8, 141.3, 138.4, 138.2, 134.1, 132.3, 129.4, 127.7, 127.1, 126.8, 123.1, 122.5, 117.3, 97.4, 54.9, 41.3, 36.9, 33.4, 32.9, 32.7, 28.8, 26.1, 25.8 (2x) ppm; MS (ESI pos.): *m/z* (%) = 647.13 (4) ([*M*+*H*]⁺, calcd. 647.23), 354.99 (19) ([*M*_{fr.I}]⁺, calcd. 355.10), 329.02 (100) ([*M*_{fr.II}]⁺, calcd. 329.10); HRMS (FTMS + p MALDI): *m/z* = 669.2099 [*M*+*Na*]⁺, calcd. for [C₃₄H₃₆ClN₆NaO₃]⁺ = 669.2124.

5.1.124 (*S*)-5-Amino-*N*-(4-((4-cyclohexyl-1-((3,5-dibromophenyl)amino)-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (155).

Compound **155** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-2-amino-4-cyclohexyl-*N*-(3,5-dibromophenyl)butanamide (**123**, 251 mg, 0.60 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 1:1 → 1:3 → 1:20) and further recrystallized from acetone to afford 79 mg (0.11 mmol, 18%) of **155**. TLC: R_F = 0.67 (SiO₂, cyclohexane/EtOAc 1:3); HPLC: 18.8 min; ¹H-NMR (600 MHz, DMSO-*d*₆, 300 K): δ = 10.39 (s, 1H), 8.57 (d, ³*J* = 7.6 Hz, 1H), 8.51 (t, ³*J* = 5.6 Hz, 1H), 7.99 (s, 1H), 7.92–7.84 (m, 4H), 7.60–7.48 (m, 5H), 7.42–7.35 (m, 3H), 6.37 (br s, 2H),

4.52–4.42 (m, 3H), 1.88–1.75 (m, 2H), 1.72–1.55 (m, 5H), 1.38–1.30 (m, 1H), 1.27–1.06 (m, 5H), 0.91–0.81 (m, 2H) ppm; ^{13}C -NMR (75 MHz, DMSO- d_6 , 300 K): δ = 171.9, 166.5, 164.1, 149.2, 143.8, 141.6, 138.4, 138.2, 132.3, 129.3, 127.8, 127.6, 127.0, 126.8, 123.1, 122.3, 120.5, 97.4, 54.9, 41.3, 36.9, 33.3, 32.8, 32.7, 28.8, 26.1, 25.8 (2x) ppm; MS (ESI pos.): m/z (%) = 737.14 (84) ($[\text{M}+\text{H}]^+$, calcd. 737.13), 486.21 (100) ($[\text{M}_{\text{fr.1}}]^+$, calcd. 486.25); HRMS (FTMS + p MALDI): m/z = 737.1271 $[\text{M}+\text{H}]^+$, calcd. for $[\text{C}_{34}\text{H}_{37}\text{Br}_2\text{N}_6\text{O}_3]^+ = 737.1273$.

5.1.125 (*S*)-5-Amino-*N*-(4-((1-(benzylamino)-4-cyclohexyl-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (156).

Compound **156** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 150 mg, 0.45 mmol) and (*S*)-2-amino-*N*-benzyl-4-cyclohexylbutanamide (**124**, 147 mg, 0.54 mmol). The crude product was triturated with cyclohexane/EtOAc (1:1) to afford 202 mg (0.34 mmol, 76%) of **156**. TLC: R_F = 0.67 (SiO₂, EtOAc/MeOH 10:1); HPLC: 15.6 min; ^1H -NMR (500 MHz, DMSO- d_6 , 300 K): δ = 8.70 (t, 3J = 6.1 Hz, 1H); 8.62 (t, 3J = 6.0 Hz, 1H), 8.49 (d, 3J = 8.1 Hz, 1H), 8.07 (s, 1H), 7.88 (d, 3J = 8.3 Hz, 2H), 7.59–7.49 (m, 4H), 7.41–7.35 (m, 3H), 7.31–7.35 (m, 5H), 6.37 (br s, 2H), 4.49–4.38 (m, 3H), 4.33–4.22 (m, 2H), 1.86–1.53 (m, 7H) 1.33–1.03 (m, 6H), 0.88–0.75 (m, 2H) ppm; ^{13}C -NMR (126 MHz, DMSO- d_6 , 300 K): δ = 172.1, 166.1, 164.2, 149.2, 143.7, 139.6, 138.6, 132.6, 129.4, 128.2, 127.6, 127.1, 126.8, 126.7, 123.1, 97.5, 53.9, 42.0, 41.3, 36.8, 33.3, 32.9, 29.2, 26.2 (2x) ppm; MS (MALDI pos.): m/z (%) = 614.8 (100) ($[\text{M}+\text{Na}]^+$, calcd. 615.31), 592.86 (55) ($[\text{M}]^+$, calcd. 592.32); HRMS (FTMS + p MALDI): m/z = 631.2784 $[\text{M}+\text{K}]^+$, calcd. for $[\text{C}_{35}\text{H}_{40}\text{KN}_6\text{O}_3]^+ = 631.2799$.

5.1.126 (*S*)-5-Amino-*N*-(4-((1-((4-chlorobenzyl)amino)-4-cyclohexyl-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (157).

Compound **157** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-2-amino-*N*-(4-chlorobenzyl)-4-cy-

clohexylbutanamide (**125**, 221 mg, 0.74 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 1:1 → 1:4 → 1:7 → EtOAc) to afford 203 mg (0.32 mmol, 54%) of **157**. TLC: R_F = 0.66 (SiO₂, EtOAc/MeOH 10:1); HPLC: 16.1 min; ¹H-NMR (500 MHz, DMSO-d₆, 300 K): δ = 8.57–8.44 (m, 2H), 8.39 (d, ³ J = 7.9 Hz, 1H), 8.00 (s, 1H), 7.88 (d, ³ J = 8.3 Hz, 2H), 7.61–7.49 (m, 4H), 7.42–7.33 (m, 5H), 7.28 (d, ³ J = 8.4 Hz, 2H) 6.39 (br s, 2H), 4.48 (d, ³ J = 6.0 Hz, 2H), 4.44–4.37 (m, 1H), 4.27 (d, ³ J = 6.3 Hz, 2H) 1.86–1.53 (m, 7H), 1.28–1.04 (m, 6H), 0.90–0.73 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-d₆, 300 K): δ = 172.1, 166.2, 164.1, 149.2, 143.6, 138.6, 138.4, 138.2, 132.6, 131.2, 129.4, 129.0, 128.1, 127.6, 127.1, 126.8, 123.1, 97.4, 53.6, 41.4, 36.7, 33.3, 32.9, 32.7, 29.1, 26.2, 25.8 ppm; MS (ESI pos.): m/z (%) = 627.35 (100) ([M+H]⁺, calcd. 627.29), 486.25 (23) ([M_{fr.I}]⁺, calcd. 486.25); HRMS (FTMS + p MALDI): m/z = 649.2661 [M+Na]⁺, calcd. for [C₃₅H₃₉ClN₆NaO₃]⁺ = 649.2670.

5.1.127 (*S*)-5-Amino-*N*-(4-((1-((4-chlorophenethyl)amino)-4-cyclohexyl-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (**158**).

Compound **158** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-2-amino-*N*-(4-chlorophenethyl)-4-cyclohexylbutanamide (**130**, 238 mg, 0.74 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 1:6 → EtOAc) to afford 250 mg (0.39 mmol, 66%) of **158**. TLC: R_F = 0.22 (SiO₂, cyclohexane/EtOAc 1:6); HPLC: 16.3 min; ¹H-NMR (500 MHz, DMSO-d₆, 300 K): δ = 8.52 (t, ³ J = 6.1 Hz, 1H), 8.27 (d, ³ J = 8.0 Hz, 1H), 8.01–7.94 (m, 2H), 7.85 (d, ³ J = 8.3 Hz, 2H), 7.60–7.49 (m, 4H), 7.42–7.35 (m, 3H), 6.39 (br s, 2H), 4.48 (d, ³ J = 6.1 Hz, 2H), 4.35–4.27 (m, 1H), 3.41–3.32 (m, 1H), 3.28–3.20 (m, 1H), 2.71 (t, ³ J = 7.0 Hz, 2H), 1.69–1.53 (m, 7H), 1.28–1.01 (m, 6H), 0.89–0.73 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-d₆, 300 K): δ = 171.8, 166.0, 164.1, 149.2, 143.6, 138.4 (2x), 138.2, 132.6, 130.7, 130.6, 129.4, 128.1, 127.6, 127.1, 126.8, 123.1, 97.4, 53.7, 41.3, 36.9, 34.3, 33.3, 32.9, 32.7, 29.3, 26.2, 25.9, 25.8 ppm; MS (ESI pos.): m/z (%) = 641.34 (29) ([M+H]⁺, calcd. 641.30),

486.23 (10) ($[M_{fr.I}]^+$, calcd. 486.25), 190.35 (100) ($[M_{fr.II}+Na]^+$, calcd. 190.13); HRMS (FTMS + p MALDI): m/z = 663.2821 $[M+Na]^+$, calcd. for $[C_{36}H_{41}ClN_6NaO_3]^+$ = 663.2821.

5.1.128 (S)-5-Amino-N-(4-((4-cyclohexyl-1-oxo-1-((pyridin-4-ylmethyl)amino)butan-2-yl)carbamoyl)benzyl)-1-phenyl-1H-pyrazole-4-carboxamide (159).

Compound **159** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1H-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (S)-2-amino-4-cyclohexyl-N-(pyridin-4-ylmethyl)butanamide (**126**, 202 mg, 0.74 mmol). The crude product was purified by column chromatography on silica (EtOAc/MeOH 10:1) to afford 153 mg (0.26 mmol, 43%) of **159**. TLC: R_F = 0.21 (SiO₂, EtOAc/MeOH 10:1); HPLC: 10.5 min; ¹H-NMR (300 MHz, DMSO-d₆, 300 K): δ = 8.58 (t, ³ J = 5.8 Hz, 1H), 8.53–8.46 (m, 3H), 8.41 (d, ³ J = 7.6 Hz, 1H), 7.98 (s, 1H), 7.88 (d, ³ J = 8.5 Hz, 2H), 7.60–7.48 (m, 4H), 7.43–7.33 (m, 3H), 7.27–7.23 (m, 2H), 6.37 (s, 2H), 4.51–4.37 (m, 3H), 4.31 (d, ³ J = 5.9 Hz, 2H), 1.90–1.55 (m, 7H), 1.35–1.06 (m, 6H), 0.94–0.75 (m, 2H) ppm; ¹³C-NMR (75 MHz, DMSO-d₆, 300 K): δ = 172.3, 166.3, 164.1, 149.4, 149.2, 148.6, 143.6, 138.4, 138.2, 129.4, 127.6, 127.1, 126.8, 123.1, 122.0, 97.4, 53.8, 41.3, 41.1, 36.7, 33.3, 32.9, 32.7, 28.9, 26.1, 25.8 (2x) ppm; MS (ESI pos.): m/z (%) = 594.35 (100) ($[M+H]^+$, calcd. 594.32), 297.81 (35) ($[M_{fr.}+Na]^+$, calcd. 297.18); HRMS (FTMS + p MALDI): m/z = 594.3188 $[M+H]^+$, calcd. for $[C_{34}H_{40}N_7O_3]^+$ = 594.3193.

5.1.129 (S)-5-Amino-N-(4-((4-cyclohexyl-1-oxo-1-((pyridin-3-ylmethyl)amino)butan-2-yl)carbamoyl)benzyl)-1-phenyl-1H-pyrazole-4-carboxamide (160).

Compound **160** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1H-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 150 mg, 0.45 mmol) and (S)-2-amino-4-cyclohexyl-N-(pyridin-3-ylmethyl)butanamide (**127**, 147 mg, 0.54 mmol). The crude product was purified by column chromatography on silica (EtOAc/MeOH 10:1) to afford 64 mg (0.11 mmol, 24%) of **160**. TLC: R_F = 0.18 (SiO₂, EtOAc/MeOH 10:1); HPLC: 10.98 min; ¹H-NMR (300 MHz, DMSO-d₆, 300 K): δ = 8.57–8.45 (m, 3H), 8.43 (dd, ³ J = 4.8, 1.8 Hz, 1H), 8.38 (d, ³ J = 7.9 Hz, 1H), 7.98 (s, 1H), 7.87 (d, ³ J = 8.3 Hz, 2H), 7.65 (dt, ³ J = 7.9, 1.9 Hz, 1H), 7.60–7.49 (m, 4H), 7.43–7.30 (m, 4H), 6.37 (s, 2H), 4.48 (d, ³ J = 5.8 Hz, 2H), 4.45–

4.35 (m, 1H), 4.31 (d, $^3J = 5.6$ Hz, 2H), 1.89–1.53 (m, 7H), 1.32–1.03 (m, 6H), 0.94–0.74 (m, 2H) ppm; ^{13}C -NMR (75 MHz, DMSO- d_6 , 300 K): $\delta = 172.1, 166.2, 164.1, 149.2, 148.6, 148.0, 143.6, 138.4, 138.2, 135.0, 134.9, 132.6, 129.4, 127.6, 127.1, 126.8, 123.3, 123.1, 97.4, 53.8, 41.3, 36.7, 33.3, 32.9, 32.6, 29.0, 26.1, 25.8$ (2x) ppm; MS (ESI pos.): m/z (%) = 594.37 (100) ($[\text{M}+\text{H}]^+$, calcd. 594.13), 319.16 (11) ($[\text{M}_{\text{fr.I}}]^+$, calcd. 319.12), 297.79 (64) ($[\text{M}_{\text{fr.II}}+\text{Na}]^+$, calcd. 297.17); HRMS (FTMS + p MALDI): $m/z = 616.3010$ $[\text{M}+\text{Na}]^+$, calcd. for $[\text{C}_{34}\text{H}_{39}\text{N}_7\text{NaO}_3]^+ = 616.3012$.

5.1.130 (*S*)-5-Amino-*N*-(4-((4-cyclohexyl-1-oxo-1-((pyridin-2-ylmethyl)amino)butan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (161).

Compound **161** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-2-amino-4-cyclohexyl-*N*-(pyridin-2-ylmethyl)butanamide (**128**, 202 mg, 0.74 mmol). The crude product was purified by column chromatography on silica (EtOAc/MeOH 10:1) to afford 183 mg (0.31 mmol, 52%) of **161**. TLC: $R_F = 0.34$ (SiO_2 , EtOAc/MeOH 10:1); HPLC: 12.5 min; ^1H -NMR (500 MHz, DMSO- d_6 , 300 K): $\delta = 8.58$ (t, $^3J = 5.9$ Hz, 1H), 8.52 (t, $^3J = 6.1$ Hz, 1H), 8.49–8.46 (m, 1H), 8.43 (d, $^3J = 7.9$ Hz, 1H), 7.99 (s, 1H), 7.88 (d, $^3J = 8.2$ Hz, 2H), 7.74 (dt, $^3J = 7.7, 1.8$ Hz, 1H), 7.59–7.49 (m, 4H), 7.42–7.36 (m, 3H), 7.29 (d, $^3J = 7.8$ Hz, 1H), 7.26–7.22 (m, 1H), 6.38 (s, 2H), 4.50–4.41 (m, 3H), 4.37 (d, $^3J = 6.1$ Hz, 2H), 1.90–1.55 (m, 7H), 1.30–1.05 (m, 6H), 0.89–0.78 (m, 2H) ppm; ^{13}C -NMR (126 MHz, DMSO- d_6 , 300 K): $\delta = 172.2, 166.3, 164.1, 158.7, 149.2, 148.8, 143.6, 138.2, 136.7, 132.6, 129.4, 127.6, 127.1, 126.8, 123.1, 122.0, 120.7, 97.4, 53.8, 44.4, 41.3, 36.8, 33.4, 32.9, 32.7, 29.0, 26.2, 25.8$ (2x) ppm; MS (ESI pos.): m/z (%) = 594.37 (100) ($[\text{M}+\text{H}]^+$, calcd. 594.32); HRMS (FTMS + p MALDI): $m/z = 616.3007$ $[\text{M}+\text{Na}]^+$, calcd. for $[\text{C}_{34}\text{H}_{39}\text{N}_7\text{NaO}_3]^+ = 616.3012$.

5.1.131 (*S*)-5-Amino-*N*-(4-((4-cyclohexyl-1-oxo-1-((thiophen-2-ylmethyl)amino)butan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (162).

Compound **162** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-2-amino-4-cyclohexyl-*N*-(thiophen-2-ylmethyl)butanamide (**129**, 370 mg, 0.74 mmol). The crude product was triturated with cyclohexane/CH₂Cl₂ (1:1) to afford 111 mg (0.19 mmol, 31%) of **162**. TLC: *R*_F = 0.67 (SiO₂, EtOAc/MeOH 10:1); HPLC: 15.3 min; ¹H-NMR (500 MHz, DMSO-*d*₆, 300 K): δ = 8.58 (t, ³*J* = 5.9 Hz, 1H), 8.52 (t, ³*J* = 6.1 Hz, 1H), 8.36 (d, ³*J* = 8.1 Hz, 1H), 7.99 (s, 1H), 7.87 (d, ³*J* = 8.3 Hz, 2H), 7.59–7.50 (m, 4H), 7.41–7.32 (m, 4H), 6.97–6.92 (m, 2H), 6.38 (br s, 2H), 4.53–4.35 (m, 5H), 1.85–1.75 (m, 1H), 1.74–1.55 (m, 6H), 1.28–1.04 (m, 6H), 0.89–0.76 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆, 300 K): δ = 171.8, 166.1, 164.1, 149.2, 143.6, 142.5, 138.4, 138.2, 132.6, 129.4, 127.6, 127.1, 126.8, 126.6, 125.2, 125.0, 123.1, 97.4, 53.6, 41.3, 37.2, 36.8, 33.3, 32.9, 29.2, 26.2, 25.8 (2x) ppm; MS (MALDI pos.): *m/z* (%) = 620.76 (100) ([*M*+Na]⁺, calcd. 621.26), 598.79 (52) ([*M*]⁺, calcd. 598.27); HRMS (FTMS + p MALDI): *m/z* = 599.2781 [*M*+H]⁺, calcd. for [C₃₃H₃₉N₆O₃S]⁺ = 599.2799.

5.1.132 (*S*)-5-Amino-*N*-(4-((1-(4-(4-chlorophenyl)piperazin-1-yl)-4-cyclohexyl-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (**163**).

Compound **163** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-2-amino-1-(4-(4-chlorophenyl)piperazin-1-yl)-4-cyclohexylbutan-1-one (**132**, 279 mg, 0.77 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 1:10 → 1:20 → EtOAc) to afford 99 mg (0.15 mmol, 24%) of **163**. TLC: *R*_F = 0.45 (SiO₂, cyclohexane/ EtOAc 1:20); HPLC: 17.4 min; ¹H-NMR (600 MHz, DMSO-*d*₆, 300 K): δ = 8.55 (d, ³*J* = 8.1 Hz, 1H), 8.49 (t, ³*J* = 5.8 Hz, 1H), 7.98 (s, 1H), 7.86 (d, ³*J* = 8.1 Hz, 2H), 7.57 (d, ³*J* = 7.9 Hz, 2H), 7.53 (t, ³*J* = 7.6 Hz, 2H), 7.41–7.35 (m, 3H), 7.23 (d, ³*J* = 8.8 Hz, 2H), 6.95 (d, ³*J* = 8.4 Hz, 2H), 6.37 (br s, 2H), 4.92–4.85 (m, 1H), 4.47 (d, ³*J* = 5.8 Hz, 2H), 3.77–3.54 (m, 4H), 3.23–3.02 (m, 4H), 1.80–1.55 (m, 7H), 1.29–1.04 (m, 6H), 0.90–0.79 (m, 2H) ppm; ¹³C-NMR (150 MHz, DMSO-*d*₆, 300 K): δ = 169.9, 165.8, 164.5, 149.5, 149.1, 143.6, 138.3, 138.2, 132.4, 129.3, 128.6, 127.5, 127.0, 126.8, 123.0, 122.8, 117.2, 97.4, 49.3, 48.5, 48.0, 44.6, 41.3, 40.1, 36.9, 33.0,

32.8, 32.7, 28.8, 26.1, 25.7 (2x) ppm; MS (ESI pos.): m/z (%) = 682.40 (93) ($[M+H]^+$, calcd. 682.33), 486.28 (41) ($[M_{fr.I}]^+$, calcd. 486.25), 392.26 (31) ($[M_{fr.II}+Na]^+$, calcd. 392.23), 324.26 (100) ($[M_{fr.III}+Na]^+$, calcd. 324.18); HRMS (FTMS + p MALDI): m/z = 704.3092 $[M+Na]^+$, calcd. for $[C_{38}H_{44}ClN_7NaO_3]^+$ = 704.3092.

5.1.133 (*S*)-5-Amino-*N*-(4-((1-(4-(4-bromophenyl)piperazin-1-yl)-4-cyclohexyl-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (**164**).

Compound **164** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-2-amino-1-(4-(4-bromophenyl)piperazin-1-yl)-4-cyclohexylbutan-1-one (**133**, 290 mg, 0.71 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc 10:1 → 1:3 → 1:20 → EtOAc) to afford 277 mg (0.38 mmol, 64%) of **164**. TLC: R_F = 0.48 (SiO₂, cyclohexane/EtOAc 1:20); HPLC: 17.6 min; ¹H-NMR (500 MHz, DMSO-*d*₆, 300 K): δ = 8.57 (d, ³*J* = 8.0 Hz, 1H), 8.51 (t, ³*J* = 6.0 Hz, 1H), 7.99 (s, 1H), 7.86 (d, ³*J* = 8.3 Hz, 2H), 7.61–7.48 (m, 4H), 7.43–7.31 (m, 5H), 6.90 (d, ³*J* = 9.1 Hz, 2H), 6.39 (br s, 2H), 4.92–4.85 (m, 1H), 4.47 (d, ³*J* = 5.9 Hz, 2H), 3.76–3.51 (m, 4H), 3.24–3.01 (m, 4H), 1.81–1.54 (m, 7H), 1.29–1.03 (m, 6H), 0.91–0.78 (m, 2H) ppm; ¹³C-NMR (126 MHz, DMSO-*d*₆, 300 K): δ = 170.0, 165.8, 164.1, 149.9, 149.2, 143.7, 138.4, 138.2, 132.4, 131.6, 129.4, 127.6, 127.1, 126.8, 123.1, 117.7, 110.5, 97.4, 49.4, 48.4, 47.9, 44.6, 41.3, 37.0, 33.2, 32.9, 32.7, 28.9, 26.2, 25.8 (2x) ppm; MS (ESI pos.): m/z (%) = 728.43 (91) ($[M+H]^+$, calcd. 728.28), 486.33 (84) ($[M_{fr.I}]^+$, calcd. 486.25), 241.24 (100) ($[M_{fr.II}+2H]^+$, calcd. 241.04), 267.14 (73) ($[M_{fr.III}]^+$, calcd. 267.01), 458.37 (19) ($[M_{fr.IV}]^+$, calcd. 458.37); HRMS (FTMS +p MALDI): m/z = 750.2548 $[M+Na]^+$, calcd. for $[C_{38}H_{44}BrN_7NaO_3]^+$ = 750.2566, 766.2304 $[M+K]^+$, calcd. for $[C_{38}H_{44}BrN_7KO_3]^+$ = 766.2306.

5.1.134 (*S*)-5-Amino-*N*-(4-((1-(4-(benzo[*d*]thiazol-2-yl)piperazin-1-yl)-4-cyclohexyl-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (**165**).

Compound **165** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-2-amino-1-(4-(benzo[*d*]thiazol-2-

yl)piperazin-1-yl)-4-cyclohexylbutan-1-one (**134**, 276 mg, 0.71 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc) to afford 315 mg (0.45 mmol, 75%) of **165**. TLC: R_F = 0.48 (cyclohexane/EtOAc 1:20); HPLC: 16.6 min; $^1\text{H-NMR}$ (400 MHz, DMSO- d_6 , 300 K): δ = 8.58 (d, 3J = 8.0 Hz, 1H), 8.50 (t, 3J = 6.0 Hz, 1H), 7.98 (s, 1H), 7.87 (d, 3J = 8.3 Hz, 2H), 7.77 (d, 3J = 7.9 Hz, 1H), 7.60–7.44 (m, 5H), 7.42–7.33 (m, 3H), 7.28 (t, 3J = 7.8 Hz, 1H), 7.08 (t, 3J = 7.8 Hz, 1H), 6.37 (br s, 2H), 4.95–4.85 (m, 1H), 4.47 (d, 3J = 5.9 Hz, 2H), 3.86–3.44 (m, 8H), 1.83–1.53 (m, 7H), 1.30–1.02 (m, 6H), 0.92–0.77 (m, 2H) ppm; $^{13}\text{C-NMR}$ (101 MHz, DMSO- d_6 , 300 K): δ = 170.0, 168.1, 165.9, 164.1, 152.3, 149.2, 143.7, 138.4, 138.2, 132.3, 130.4, 129.3, 127.6, 127.0, 126.8, 123.1, 121.4, 121.2, 118.7, 97.4, 49.5, 41.3, 37.0, 33.1, 32.9, 32.7, 28.8, 26.1, 25.8 ppm; MS (MALDI pos.): m/z (%) = 704.35 (100) ($[\text{M}]^+$, calcd. 704.33), 705.36 (39) ($[\text{M}]^+$, calcd. 705.33), 726.32 (22) ($[\text{M}+\text{Na}-\text{H}]^+$, calcd. 726.31), 727.32 (14) ($[\text{M}+\text{Na}-\text{H}]^+$, calcd. 727.31); HRMS (FTMS +p MALDI): m/z = 705.3327 $[\text{M}+\text{H}]^+$, calcd. for $[\text{C}_{39}\text{H}_{48}\text{N}_8\text{O}_3\text{S}]^+ = 705.3330$.

5.1.135 (*S*)-5-Amino-*N*-(4-((1-(4-(benzo[*d*]oxazol-2-yl)piperazin-1-yl)-4-cyclohexyl-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1*H*-pyrazole-4-carboxamide (**166**).

Compound **166** was prepared according to general procedure F using 4-((5-amino-1-phenyl-1*H*-pyrazole-4-carboxamido)methyl)benzoic acid (**70**, 200 mg, 0.60 mmol) and (*S*)-2-amino-1-(4-(benzo[*d*]oxazol-2-yl)piperazin-1-yl)-4-cyclohexylbutan-1-one (**135**, 265 mg, 0.71 mmol). The crude product was purified by column chromatography on silica (cyclohexane/EtOAc) to afford 247 mg (0.36 mmol, 60%) of **166**. TLC: R_F = 0.38 (cyclohexane/EtOAc 1:20); HPLC: 16.1 min; $^1\text{H-NMR}$ (400 MHz, DMSO- d_6 , 300 K): δ = 8.58 (d, 3J = 7.9 Hz, 1H), 8.50 (t, 3J = 6.0 Hz, 1H), 7.98 (s, 1H), 7.87 (d, 3J = 8.3 Hz, 2H), 7.60–7.48 (m, 4H), 7.44–7.35 (m, 4H), 7.30 (d, 3J = 7.7 Hz, 1H), 7.16 (t, 3J = 7.7 Hz, 1H), 7.03 (t, 3J = 7.7 Hz, 1H), 6.37 (br s, 2H), 4.95–4.85 (m, 1H), 4.47 (d, 3J = 5.9 Hz, 2H), 3.86–3.49 (m, 8H), 1.82–1.53 (m, 7H), 1.32–1.02 (m, 6H), 0.95–0.77 (m, 2H) ppm; $^{13}\text{C-NMR}$ (101 MHz, DMSO- d_6 , 300 K): δ = 170.3, 165.9, 164.1, 161.6, 149.2, 148.3, 143.7, 138.4, 138.2, 132.3, 129.3, 127.6, 127.0, 126.8, 124.0, 123.1, 120.7, 115.9, 109.0, 97.4, 49.5, 44.3, 41.3, 37.0, 33.1, 32.9, 32.7, 28.8, 26.1, 25.8 ppm; MS (MALDI pos.): m/z

(%) = 688.63 (100) ($[M]^+$, calcd. 688.35), 689.64 (51) ($[M]^+$, calcd. 689.35), 710.58 (8) ($[M+Na-H]^+$, calcd. 726.31), 712.58 (6) ($[M+Na-H]^+$, calcd. 712.58); HRMS (FTMS +p MALDI): m/z = 689.3544 $[M+H]^+$, calcd. for $[C_{39}H_{45}N_8O_4]^+ = 689.3558$.

5.2 Biochemical and biological methods.

5.2.1 Differential scanning fluorimetric (DSF)-assay.

Differences in the melting temperature (ΔT_m) data were measured as described in Fedorov et al. [45].

5.2.2 NanoBRET assay.

The assay was performed as described previously [38, 46]. In brief: Full-length MAPK14 ORF (Promega, NV1661) cloned in frame with a C-terminal NanoLuc-fusion were transfected into HEK293T cells using FuGENE HD (Promega, E2312) and proteins were allowed to express for 20h. Serially diluted inhibitor and NanoBRET Kinase Tracer K4 (Promega, N2540) at 100 nM were pipetted into white 384-well plates (Greiner 781 207) using an Echo acoustic dispenser (Labcyte). The corresponding MAPK14-transfected cells were added and reseeded at a density of 2×10^5 cells/mL after trypsinization and resuspending in Opti-MEM without phenol red (Life Technologies). The system was allowed to equilibrate for 2 hours at 37°C/5% CO₂ prior to BRET measurements. To measure BRET, NanoBRET NanoGlo Substrate + Extracellular NanoLuc Inhibitor (Promega, N2540) was added as per the manufacturer's protocol, and filtered luminescence was measured on a PHERAstar plate reader (BMG Labtech) equipped with a luminescence filter pair (450 nm BP filter (donor) and 610 nm LP filter (acceptor)). Competitive displacement data were then plotted using GraphPad Prism 8 software using a 4-parameter curve fit with the following equation: $Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogIC}_{50} - X) * \text{HillSlope}))}$

5.2.3 Crystallization and structure determination.

Recombinant p38 α , purified as previously described [47], was incubated with the inhibitors (protein concentration = 10-13 mg/ml, inhibitor concentration = 1 mM), and the complexes were crystallized using the sitting-drop vapor diffusion method at 4 °C and PEG smears-based conditions [48] containing either

12-20% medium-molecular-weight PEG smears and 0.1 M MES pH 5.8-6.2 or 25% broad-molecular-weight PEG smears and 0.1 M MES, pH 6.0. Diffraction data were collected at Diamond i03, BESSY 14.3 and SLS X06SA, and were processed and scaled with iMOSFLM [49], XDS [50] and Scala [51], respectively. Initial structures were solved by molecular replacement using Phaser [52] and the published p38 α coordinates (PDB ID 5LAR; [23]). The final models were rebuilt in COOT [53] and refined using either REFMAC [54] or PHENIX [55]. The data collection and refinement statistics are summarized in Supplementary Table S3. Coordinates and structure factors of the crystal structures of the human p38 α MAP kinase in complex with MCP-081, **137**, **142**, **143**, **145**, **146**, **148**, **150**, **158**, and **159** were deposited in the Protein Data Bank under accession codes 6Y6V, 6YK7, 6Y4T, 6Y4U, 6Y4V, 6Y4W, 6YJC, 6ZWP, 6Y4X, and 6ZWR, respectively.

5.2.4 Inhibition of Hsp27 phosphorylation in HCT15 cells.

HCT15 cells were treated for 2 h with inhibitors at different concentrations or vehicle (DMSO) and then stimulated with 10 μ g/ml anisomycin (Merck, A9789) for 30 min. Levels of p-Hsp27 (antibody: Cell Signaling Technology, 2401) were determined via Western Blot analysis of whole cell lysates. Vinculin (antibody: Merck, V9131) was used as loading control. The chemiluminescence was measured with the Bio-Rad ChemiDoc MP Imaging System and recorded with the Image Lab 4.1 software for image acquisition.

5.2.5 TNF- α release assay in human whole blood.

The assay was performed as published by Bauer et al. [56]. Briefly, the tested compounds were preincubated in human whole blood from two different donors for 15 min before TNF- α release was stimulated by addition of LPS. After stimulation, the samples were incubated at 37 °C and 6% CO₂ for 2.5 h, followed by centrifugation to separate cells and plasma. The supernatant was separated, and the TNF- α levels were determined using a sandwich ELISA.

5.2.6 *In vitro* metabolism studies.

Pooled male human liver microsomes (HLM) were purchased from Xenotech. These microsomes were characterized in protein and cytochrome P-450 content. All incubations were made in the presence of an NADPH-regenerating system, consisting of 5 mM Glucose-6-phosphate, 5 U/mL Glucose-6-phosphate dehydrogenase and 1 mM NADP⁺. The substrate (100 μ M), the NADPH regenerating system and 3.8 mM MgCl₂ x 6 H₂O in 0.1 M Tris buffer (pH 7.4 at 37 °C) were pre-incubated for 5 min in a shaking heating block at 37 °C and 650 rpm. The incubation mix was split into 50 μ L aliquots, and the reaction was started by addition of the HLM. Thereby the microsomal protein content was standardized to 1 mg/mL. To follow the course of metabolism, the reaction tubes were quenched at selected time points (0, 10, 20, 30, 60, 120, 180, and 240 min) by adding 100 μ L ice-cold internal standard at a concentration of 20 μ M in acetonitrile (ACN). The samples were vortexed for 30 s and centrifuged (19800 relative centrifugal force/4 °C/20 min). The supernatant was used directly for LC-MS analysis. All incubations were conducted in triplicate, and incubations with heat-inactivated HLM were used to show that analyte reduction results from metabolic degradation only. In all incubations, a limit of 1% organic solvent was not exceeded.

5.2.6.1 LC-MS analysis.

Chromatographic separation was performed on an Alliance 2695 Separations Module (Waters GmbH, Eschborn) using a Dr. Maisch Nucleosil 100 C18, 5 μ , 53 x 4.6 mm column with water (A) and acetonitrile (B) with 0.1% (v/v) formic acid as mobile phase. The following gradient was applied: 20% B from 0–0.2 min, 20–80% B from 0.2–5.5 min, 20% B from 5.6–14 min at a flow rate of 400 μ L/min; samples were maintained at 4 °C, the column temperature was set to 40 °C and the injection volume was 10 μ L. The detection was performed on a Waters Quattro Micro API Mass Spectrometer (Waters GmbH, Eschborn) operated in electrospray-ionization (+) mode. The spray voltage was set to 4 kV. The heated capillary operated at 270 °C, and the desolvation gas flow was set to 650 L/h.

Supporting Information

The following supplemental data are available: Table S1: DSF-assay results for the 47-kinase panel; Supporting information 1: Selectivity screening and NanoBRETTM-assay results for compound **137** and **159**; X-ray data collection and refinement statistics of p38 α -ligand complexes; Supporting information 2: NMR and HRMS spectra.

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