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Design, Synthesis, and Structure−Activity Relationship Studies of Dual Inhibitors of Soluble Epoxide Hydrolase and 5-Lipoxygenase

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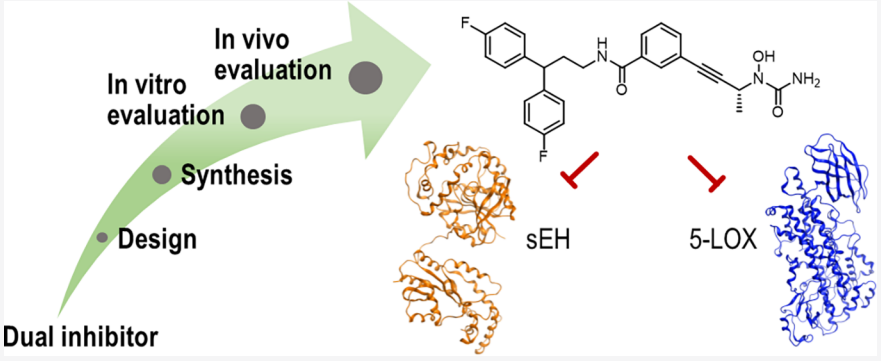

ABSTRACT: Inhibition of multiple enzymes of the arachidonic acid cascade leads to synergistic anti-inflammatory effects. Merging of 5-lipoxygenase (5-LOX) and soluble epoxide hydrolase (sEH) pharmacophores led to the discovery of a dual 5-LOX/sEH inhibitor, which was subsequently optimized in terms of potency toward both targets and metabolic stability. The optimized lead structure displayed cellular activity in human polymorphonuclear leukocytes, oral bioavailability, and target engagement in vivo and demonstrated profound anti-inflammatory and anti-fibrotic efficiency in a kidney injury model caused by unilateral ureteral obstruction in mice. These results pave the way for investigating the therapeutic potential of dual 5-LOX/sEH inhibitors in other inflammation- and fibrosis-related disease models.

INTRODUCTION

Inflammation is a complex physiological process, which is activated by the immune system upon a harmful stimulus.[1] Among others, inflammation is mediated by different metabolites of arachidonic acid (AA). AA is metabolized by a cascade of biochemical reactions, which are subdivided into three major pathways, namely, the 5-lipoxygenase (5-LOX), the cyclooxygenase (COX), and the cytochrome P450 (CYP450) pathways. Lipids generated by the 5-LOX branch are leukotrienes (LTs) and lipoxins (LXs). The LTs are pro-inflammatory and chemotactic mediators; hence, the inhibition of 5-LOX is an established strategy to counteract asthma[2] and is under intensive investigation for diverse inflammatory diseases.[3] The prostanoids produced by the COX pathway are divided into prostaglandins (PGs) and thromboxane (TX), while the metabolites of the CYP450 branch are called epoxyeicosatrienoic acids (EETs). The soluble epoxide hydrolase (sEH), located in the CYP450 branch, converts the anti-inflammatory EETs to the less biological active dihydroxyeicosatrienoic acids (DHETs).[4] Hence, inhibition of sEH leads to accumulation of EETs and pronounced anti-inflammatory effects in the settings of acute and chronic inflammation, such as rheumatoid arthritis,[5] cardiovascular disease, inflammatory bowel disease, and sepsis.[6]

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most popular anti-inflammatory drugs on the market. NSAIDs effectively target the COX pathway; however they are associated with a broad range of side effects.[7] Some of these side effects are caused by shunting of AA metabolites within the AA cascade.[8] One prominent example is acetylsalicylic acid-induced asthma caused by accumulation of non-metabolized AA, which shunts the LOX pathway, resulting in increased leukotriene E_4 (LTE_4) levels.[9] Another shunting

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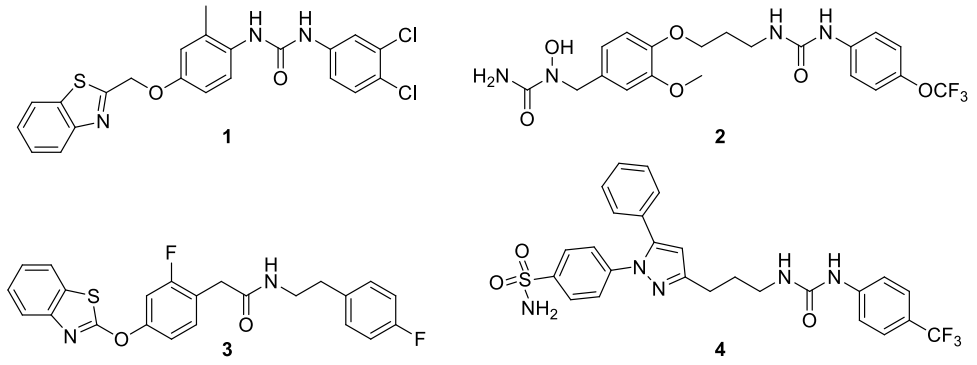

Figure 1. Dual inhibitors of the AA cascade.

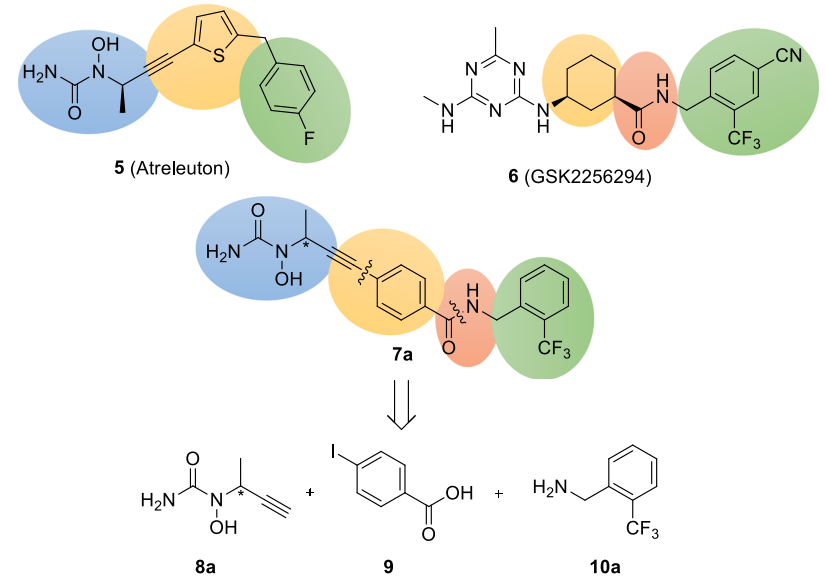

Figure 2. Design of the merged dual inhibitor **7a**. Clinical candidates **5** and **6** served as starting points and were subdivided into pharmacophore features (blue: iron chelating *N*-hydroxy urea moiety; yellow: lipophilic central core; orange: amide as epoxide mimetic; green: terminal aromatic substituent). The recombination of the essential pharmacophore features led to the design of compound **7a**, which can be retrosynthetically disconnected into three synthons (**8a**, **9**, and **10a**).

effect was reported by Jung et al.[10] showing that treatment of mice with an sEH inhibitor promoted proteinuria possibly due to the shift to higher LT levels. Shunting effects might be prevented by inhibition of more than one enzyme in the AA cascade.[11] Especially, the combination of sEH inhibition with one of the remaining branches of the AA cascade seems to be a valuable strategy to design efficient and safe anti-inflammatory compounds.[4] First evidence on these findings was reported by Liu et al., who demonstrated synergistic effects of an sEH inhibitor with either a 5-lipoxygenase activating protein (FLAP) inhibitor or COX inhibitor acetylsalicylic acid in lipopolysaccharide (LPS)-induced inflammation in mice.[12] Encouraged by these findings, researchers made several successful attempts to design multitarget ligands affecting sEH and an additional enzyme or receptor of the AA cascade.[4]

Temml et al. developed **1**,[13] a dual sEH and FLAP inhibitor, and Garsha et al. characterized the pharmacological profile and demonstrated that **1** decreased leukotriene formation in vivo in a zymosan-induced mouse peritonitis model.[14] A dual sEH/5-LOX inhibitor **2** blocked the LPS-induced adhesion of leukocytes to endothelial cells by impairing leukocyte function.[15] The dual sEH/LTA_4H inhibitor **3** reduced the leukotriene B_4 (LTB_4) and prostaglandin levels in bacteria-activated M1 and M2 macrophages.[16] A dual inhibitor of sEH and COX-2 **4**[17] exhibits excellent in vivo efficacy in murine models of cancer,[18,19] pulmonary fibrosis,[20] and allergen-induced airway inflammation[21] (Figure 1).

Encouraged by these findings, we designed an orally available dual inhibitor of 5-LOX and sEH in order to enable the investigation of this designed multitarget ligand (DML) in different inflammatory in vivo models. Previous attempts yielded DML **2**, which exhibits several drawbacks. First, the synthetic access to **2** employs a linear route with an introduction of the *N*-hydroxy urea moiety within the last three steps with mediocre yields, which hampers the exploration of structure−activity relationships. Second, the flexible linker and the urea functionality are potential drawbacks in terms of metabolic stability and solubility. Therefore, we employed a novel design strategy, incorporating the typical *N*-hydroxy urea pharmacophore of 5-LOX inhibitors as in **5**[22] and the amide moiety, which acts as an epoxide mimic in sEH inhibitors like in **6**,[23] and both compounds advanced in clinical trials. The aforementioned essential pharmacophore groups were merged at the overlapping lipophilic central core and the terminal aromatic moiety, leading to the dual ligand **7a** as a starting point for

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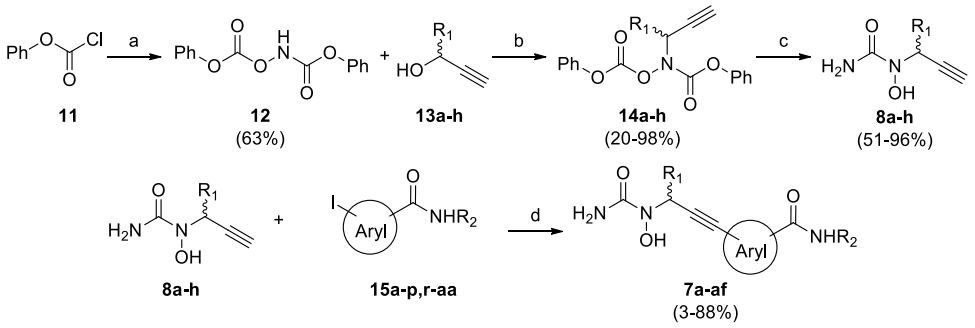
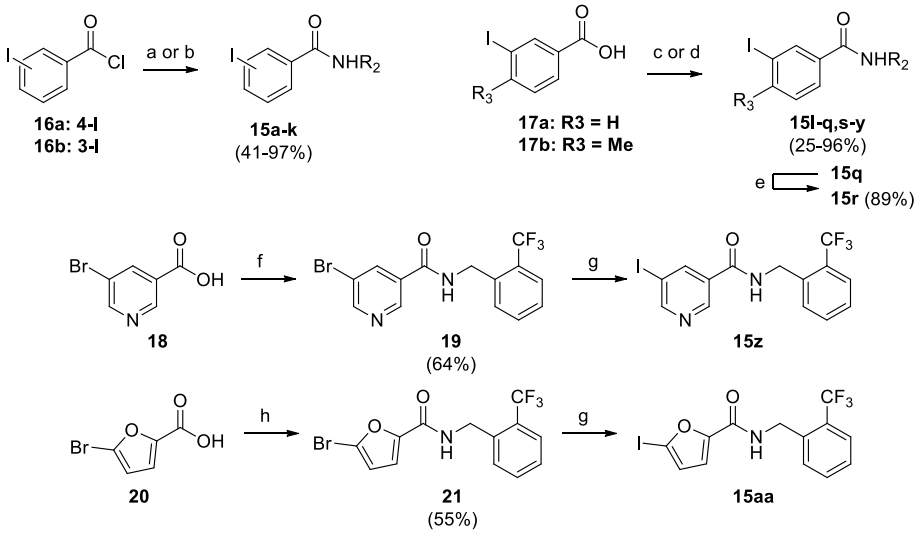
Scheme 1. Synthetic Routes to the Alkyne Derivatives 8a−h and the Target Structure 7a−af[a]



[a](a) (i) $NaHCO_3$ in water, hydroxylamine hydrochloride, 0 °C, 0.5 h, (ii) **11**, $NaHCO_3$ in water, rt, 2 h; (b) PPh_3, DIAD, dry THF, 0 °C → rt, 1 h; (c) NH_3, *tert*-butyl alcohol/THF (3:1), −78 °C → rt, 5−7 bar, 16−20 h; (d) $Pd(ACN)_2Cl_2$, PPh_3, CuI, DIPA, dry ethyl acetate, rt, 36−48 h.

Scheme 2. Preparation of Amide Intermediates 15a−aa



[a](a) DCE, DIPEA, **10a−i**, 90 °C μw, 1 h; (b) **10j**, **16b**, DCM, DIPEA, 4-DMAP, rt, 18 h; (c) **10a,k−o** PyBOP, DIPEA/DIPA, dry THF, rt, 16−20 h; (d) **10p−u**, PyBOP, $HOBt·H_2O$, dry THF, rt, 16 h /60 °C μw, 1 h; (e) methanesulfonyl chloride, NEt_3, DCM, 0 °C → reflux, 17 h; (f) (i) **18**, HBTU, dry DMF, 0 °C, (ii) **10a**, 4-methylmorpholine, EDC·HCl, rt, 72 h; (g) NaI, CuI, trans-1,2-cyclohexanediamine, dry 1,4-dioxane, 155 °C μw, 8 h; (h) **10a**, PyBOP, DIPEA, dry DMF, rt, 48 h.

exploration of the structure−activity relationship (SAR) (Figure 2). The retrosynthetic disconnection defined the distinct molecular parts for optimization: the central core, the alkyl substituent between the *N*-hydroxy urea and the alkyne linker, and the terminal aromatic substituent.

CHEMISTRY

The retrosynthetic analysis of compound **7a** suggested a convergent synthesis route consisting of two main steps; an amide coupling followed by a Sonogashira coupling (Figure 2). The alkyne derivatives **8a−h** were synthesized in a three-step procedure displayed in Scheme 1. First, phenyl chloroformate **11** and hydroxylamine hydrochloride were coupled to yield the protected *N*-hydroxy carbamate **12**, which subsequently reacted in a Mitsunobu reaction to derivatives **14a−h**. Deprotection was accomplished by ammonolysis of **14a−h** in acceptable yields under high pressure of ammonia in an autoclave. In the case of optically pure alkyne derivatives **8c,d**, the assumption was made where use of stereochemically pure reagents **13c,d** leads to intermediate **14c,d** with a defined configuration. This assumption is justified by a closer look at the mechanism of the Mitsunobu reaction, which suggests the inversion of the stereogenic center directly linked to the hydroxyl moiety. To verify this, the derivatives **8c** and **8d** were coupled with (*S*)-(+)-α-methoxyphenylacetic acid and the diastereomeric ratio (dr) was determined (dr 9:1 in both cases; experimental details in the Supporting Information). Subsequently, Sonogashira coupling was performed using alkyne derivatives **8a−h** and amides **15a−p,r−aa** to yield the final products **7a−ag**.

Starting from acyl chlorides **16a** or **16b**, the amide coupling was performed directly under microwave irradiation, except for (2-(trifluoromethoxy)phenyl)methanamine **10j**, where milder reaction conditions were required. The coupling of acids **17a**, **17b**, **18**, and **20** was performed with standard amide coupling reagents such as PyBOP and $HOBt·H_2O$ or HBTU and EDC·HCl. In order to facilitate Sonogashira coupling, halogen exchange from bromine (amide **19** and **21**) to iodine was accomplished in a similar way to the published procedure of Klapars et al.[24] (Scheme 2).

Evaluation of the inhibitory potential of inhibitors **7** on both targets showed that **7t** is the most promising compound for further optimization. To increase the metabolic stability, halogen atoms as well as a sulfonamide substituent were

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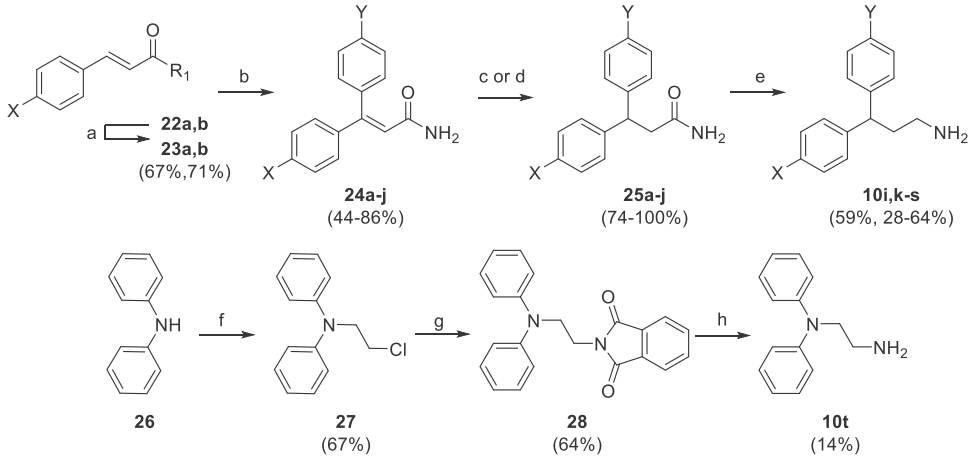
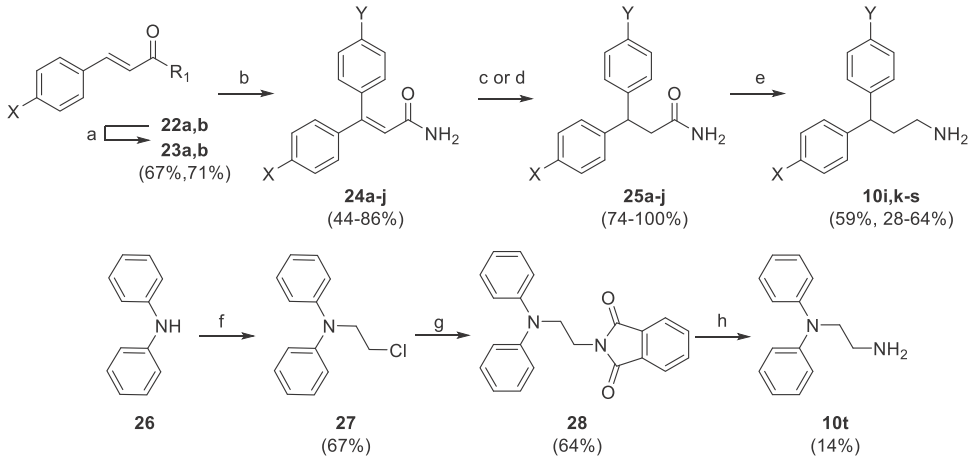
Scheme 3. Synthetic Route to Benzhydryl-Derived Amines 10j,k−s,t[a]



[a](a) (i) NEt_3, ethyl chloroformate, THF, −15 °C, 1 h, (ii) 32% ammonia in water, −15 °C → rt, 18 h; (b) iodoaryl derivative, $Pd(OAc)_2$, NEt_3, 100 °C, 16−48 h; (c) 10 wt % Pd/C, hydrogen atmosphere (balloon/autoclave), MeOH, rt, 16−48 h; (d) **24d** or **24g,** $Pd(OH)_2$, hydrogen atmosphere (balloon), EtOH, rt, 16−48 h; (e) $LiAlH_4$ in THF, dry THF, 0 °C → reflux, 2−5 h; (f) (i) chloroacetic acid, $NaBH_4$, toluene, rt, 3 h, (ii) **26**, reflux, 4 h; (g) potassium phthalimide, DMF, 140 °C μw, 1 h; (h) $N_2H_4{\cdot}H_20$, EtOH, reflux, 16 h.

Scheme 4. Synthetic Route for the Asymmetric Synthesis of Amine Precursor 10u[a]



[a](a) Oxalyl chloride, dry THF, rt, 17 h; (b) (i) (*S*)-4-*tert*-butyl-2-oxazolidinone, *n*BuLi in THF, −78 °C, (ii) **29**, DCM, −78 °C → 0 °C, 1.5 h; (c) (4-fluorophenyl)boronic acid, $Pd(OAc)_2$, bipy, MeOH/water (1:3), 80 °C, 14 h; (d) $LiBH_4$, Et_2O, 0 °C → rt, 4 h; (e) Dess−Martin periodinane, DCM, rt, 2.5 h, (f) NH_4OAc in EtOH, $NaBH(OAc)_3$, 32% ammonia in water, reflux, 13 h.

introduced to the benzhydryl moiety (Scheme 3). The substituted cinnamic acids **22a,b** were converted into the primary amides **23a,b** with ethyl chloroformate and ammonia, while the unsubstituted cinnamamide **23c** was commercially available. *β*-Arylation via the Heck reaction with palladium(II) acetate yielded **24a−j** in acceptable yields and hydrogenation (**25a-j**) followed by reduction with lithium aluminum hydride gave the amines **10j,k−s**. Furthermore, we changed the tertiary carbon atom against nitrogen (**7ae**). Therefore, in a reductive amination, amine **26** was coupled with 2-chloroacetic acid.[25] The first step of the reaction was an in situ generation of a triacyloxyborohydride analogue, which was self-reduced to the free aldehyde or a boron species with the same oxidation level as the aldehyde. The second step was the reductive amination.[26] The yielded tertiary amine **27** reacted via a Gabriel reaction to structure **28.** Using hydrazine hydrate the phthalimide derivative **28** was cleaved to yield the free amine **10t**.

To examine the influence of the second stereogenic center of the unsymmetrically substituted benzhydryl moiety, an Evans auxiliary assisted asymmetric synthesis was performed. Zhi et al.[27] published a synthetic route for the asymmetric synthesis of (*R*)-tolterodine, which was adapted for the synthesis of **10u** (Scheme 4). First, cinnamic acid **22a** was converted into acyl chloride **29** and coupled with the Evans auxiliary. Second, **30** was linked with a boronic acid to compound **31** with palladium catalysis. The auxiliary was cleaved with $LiBH_4$ and the resulted alcohol **32** was oxidized to the aldehyde **33** with Dess−Martin periodinane. The last step was a reductive amination with $NaBH(OAc)_3$ and ammonia to the amine **10u**. Amide coupling and Sonogashira coupling led to the (*R,R*)-compound **7af** (Schemes 1 and 2). The (*S,R*)-diastereomer **7ag** was obtained via chiral chromatographic separation of **7ab**.

RESULTS AND DISCUSSION

Inhibition of sEH and 5-LOX. The inhibitory potency on both targets was evaluated using enzyme activity assays with

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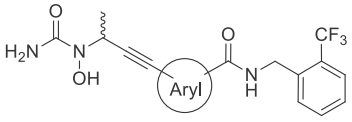
recombinantly expressed proteins. For sEH, the conversion of the fluorogenic substrate PHOME (3-phenyl-cyano(6-methoxy-2-naphthalenyl)methyl ester-2-oxiraneacetic acid) was monitored.[28] The enzymatic activity of 5-LOX was accessed using the endogenous substrate AA and monitoring the product formation by HPLC.[29] The prototype inhibitor **7a** yielded promising IC_{50} values of 2.6 μM (sEH) and 1.0 μM (5-LOX) and served as a starting point for further optimization. By changing the substitution pattern of the central core from para to meta (compound **7b**), the inhibitory potency toward sEH was almost 30-fold increased, together with a slightly better IC_{50} value for 5-LOX. An introduction of a heterocyclic moiety as well as a methyl substituent (compounds **7c–e**) resulted in a loss of potency on both targets, suggesting that a plain 1,3-substituted phenyl core serves best for further optimization efforts (Table 1).

Table 1. In Vitro Inhibitory Activity of the Dual sEH/5-LOX Inhibitors: Variation of the Central Aromatic Core

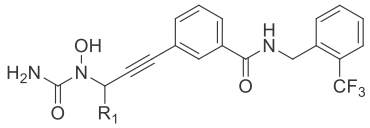


No.	Aryl	Inhibition sEH IC_{50} [μM][a]	Inhibition 5-LOX IC_{50} [μM][b]
5	-	2.7 (2.3-3.1)	0.09 (0.07-0.12)
6	-	0.0007 (0.0006-0.0008)	n.d.
7a		2.6 (1.9-3.8)	1.0 (0.8-1.3)
7b		0.09 (0.08-0.09)	0.7 (0.5-0.9)
7c		0.5 (0.5-0.6)	10 (8-13)
7d		0.6 (0.5-0.6)	4 (3-5)
7e		3.1 (2.9-3.3)	1.7 (1.2-2.4)

[a]All values were measured at least thrice in triplicate ($n \geq 3$) using recombinantly expressed protein. A 95% confidence interval is displayed in brackets. [b]All values were measured at least thrice ($n \geq 3$). A 95% confidence interval is displayed in brackets, n.d. = not determined.

Next optimization steps concerned the alkyl chain adjacent to the *N*-hydroxyurea moiety. The alkyl chain elongation was favored by both enzymes, while the introduction of a hydrogen (**7f**) led to loss of potency toward 5-LOX. Unbranched chains (**7i,j**) were slightly better tolerated by 5-LOX, while the opposite was observed for sEH. The influence of the stereochemistry was examined with the methyl derivative. The alcohol precursors **13c** and **13d** were commercially available in both orientations and coupled, as shown in Scheme 1. The *R*-derivative **7h** inhibited that 5-LOX slightly better than the *S*-derivative **7g**. In general, the length of the alkyl chain has only small effects on the inhibitory potency toward 5-LOX; therefore, further optimization steps were performed with the *R*-methyl substituent, which yielded satisfactory submicromolar IC_{50} values toward both enzymes (Table 2).

Table 2. Influence of the Alkyl Chain toward the Inhibitory Potential



No.	R_1	Inhibition sEH IC_{50} [μM][a]	Inhibition 5-LOX IC_{50} [μM][b]
7f	H	0.05 (0.04-0.05)	4 (3-5)
7g	Me (*S*)	0.06 (0.05-0.07)	0.7 (0.4-1.3)
7h	Me (*R*)	0.06 (0.05-0.06)	0.6 (0.4-0.9)
7i[c]	Et	0.022 (0.021-0.024)	0.5 (0.4-0.6)
7j[c]	*n*-Propyl	0.012 (0.011-0.013)	0.3 (0.2-0.4)
7k[c]	*i*-Propyl	0.011 (0.010-0.011)	0.5 (0.4-0.7)
7l[c]		0.005 (0.004-0.005)	0.6 (0.4-0.8)

[a]All values were measured at least thrice in triplicate ($n \geq 3$). A 95% confidence interval is displayed in brackets. [b]All values were measured at least thrice ($n \geq 3$). A 95% confidence interval is displayed in brackets, [c]Enantiomeric ratio undetermined.

The following optimization step was applied to the terminal aromatic moiety. The development of sEH inhibitors is facilitated by co-crystal structures and chemically diverse published inhibitors. In contrast, crystallographic data of 5-LOX inhibitors is absent. With these circumstances, structure-based development of a 5-LOX inhibitor is hindered, and we decided to use aromatic substituents frequently used in published sEH inhibitors and evaluated the inhibitory potential regarding both enzymes. By keeping the meta substitution at the central ring, we started to synthesize amides connected to a benzylic moiety with various substitution patterns.[30−32] In addition, we used structural motifs from our published dual inhibitors containing an ethyl linker ending in an aryl moiety (Figure 3).[16]

An ethyl linker as well as a meta substitution of the newly introduced aryl group is disfavored by both enzymes (**7m–o**). An ortho or ortho/para substitution pattern at the benzylic moiety seems to be tolerated by 5-LOX (**7p**, **7q**), but the 2,5-substitution pattern is less tolerated in the case of a trifluoromethyl group (**7r**, **7s**) (Table 3). The best IC_{50} values on both targets were achieved with a propyl linker with a

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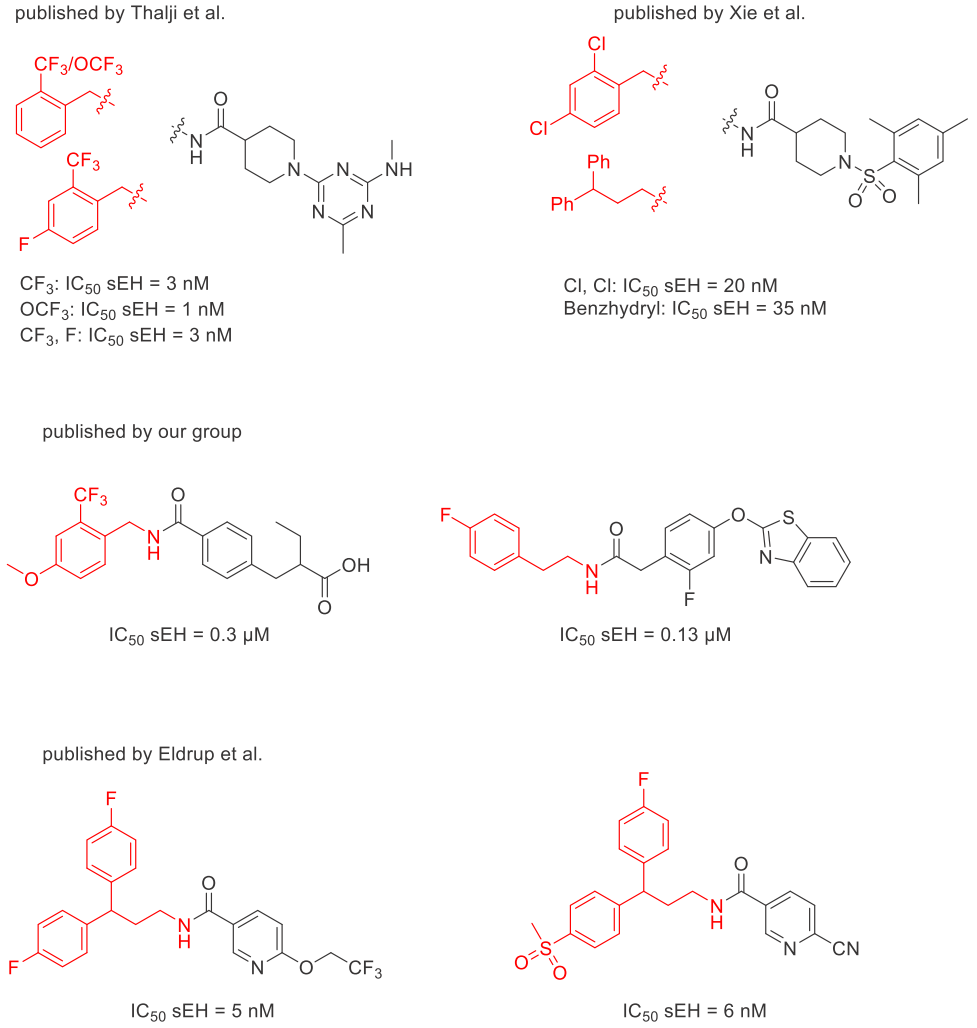

Figure 3. Lipophilic amide substituents of published sEH inhibitors.

benzhydryl group (**7t**), previously published by Xie et al.[30] and Eldrup et al.[33]

As stated in the publication by Eldrup et al., the unsubstituted benzhydryl group is prone to metabolic oxidation.[33] It also holds true for compound **7t**, of which only 27% remained after 1 h of incubation in rat liver microsomes (RLMs) (Table 5). In the work of Eldrup et al., the metabolic stability could be raised by incorporation of fluoro and sulfonamide residues (Figure 3). This strategy was also used in our work. The sulfonamide derivatives (**7y**, **7z**) were disfavored by 5-LOX; in contrast, a 3,4-dichlorophenyl ring increased the inhibitory potential (**7aa**) (Table 4). The combination of two halogenated phenyl rings gave more metabolically stable inhibitors (**4ab**−**4ag**). Regarding the sEH inhibitory activity, the differentiation between IC_{50} values of these compounds is difficult due to the resolution limit of the assay system, where a concentration of 3 nM enzyme is employed. Nevertheless, the substitution on both phenyl rings did not impair the inhibitory activity regarding the sEH, while the inhibitory activity against 5-LOX was slightly decreased. To examine the influence of the second stereogenic center between the unsymmetrically substituted benzhydryl moiety, both diastereomers (*R*,*R*-**4af** and *R*,*S*-**4ag**) were prepared and evaluated. **4af** exhibited a slightly higher inhibitory activity regarding 5-LOX.

In order to rationalize the SAR of dual sEH/5-LOX inhibitors, we co-crystallized compound **7w** in complex with the C-terminal domain of sEH (Figure 4a). Both diastereomers of **7w** fit well to the electron density in the binding site (Figure 4b). The amide moiety of **7w** acts, as intended, as an epoxide mimic and interacts via H bonds with the catalytic residues Asp335, Tyr383, and Tyr466. The adjacent aromatic core displays an edge-to-face aromatic interaction with Phe381. The preference of the 1,3-substitution pattern of the aromatic core (compound **7b**) in comparison to the 1,4-substituted compound **7a** can be explained by the shape of the lipophilic tunnel occupied by the alkyne linker. The hydroxyl urea group does not display directed interactions with the protein and is not resolved in the electron density. The benzhydryl group fits very well into the bifurcated lipophilic subpocket formed by Leu408, Leu417, Met419, Tyr383, and His524. As mentioned before, the configuration of the unsymmetrically substituted benzhydryl moiety cannot be unambiguously assigned. However, the shape and the size of the pocket suggest that one of the phenyl rings should carry small substituents like −F, while the size of the residues bound to the second phenyl ring is almost not limited.

A recent publication by Gilbert et al. demonstrated that 5-LOX possesses at least two binding pockets, which can be occupied by potent lipophilic inhibitors.[34] Therefore, a binding

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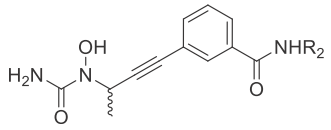
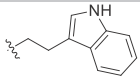
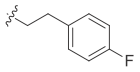
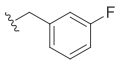
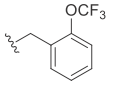
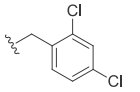
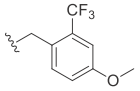
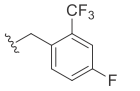
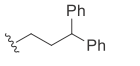
Table 3. Influence of the Terminal Aromatic Moiety toward the Inhibition of Both Enzymes



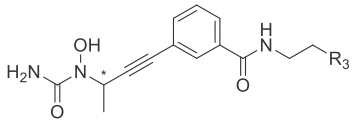
No.	R_2	Inhibition sEH IC_{50} [μM][a]	Inhibition 5-LOX IC_{50} [μM][b]
7m		3.1 (2.9-3.3)	1.7 (1.2-2.4)
7n		1.2 (1.1-1.2)	3.1 (2.2-4.4)
7o		9.0 (8.7-9.3)	4 (3-6)
7p		0.016 (0.016-0.017)	1.0 (0.8-1.2)
7q		0.04 (0.03-0.04)	0.6 (0.5-0.7)
7r		0.004 (0.004-0.005)	1.7 (1.4-2.2)
7s		0.029 (0.027-0.031)	1.3 (0.9-1.7)
7t		0.002 (0.001-0.002)	0.3 (0.3-0.4)

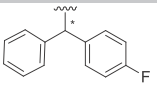
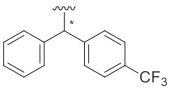
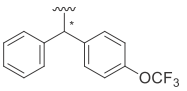
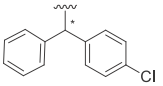
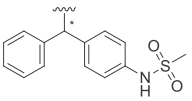
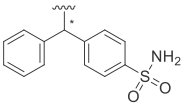
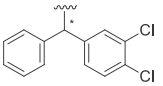
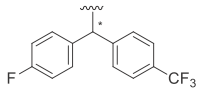
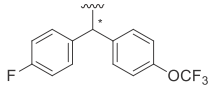
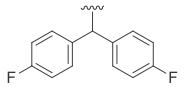
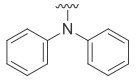
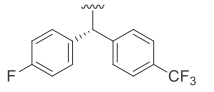
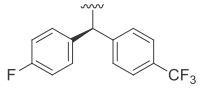
[a]All values were measured at least thrice in triplicate ($n \geq 3$). A 95% confidence interval is displayed in brackets. [b]All values were measured at least thrice ($n \geq 3$). A 95% confidence interval is displayed in brackets.

mode hypothesis based on molecular docking would be too uncertain.

In Vitro Pharmacological Characterization. The symmetric substitution with two fluorine atoms (**7ad**) avoided a second chiral center and was tolerated by both enzymes. Another strategy to circumvent the second stereogenic center was the replacement of the carbon atom between both phenyl groups by nitrogen (compound **7ae**). This modification also gained similar IC_{50} values to **7ad**, but the metabolic stability of **7ae** in RLMs is very low (Table 5). The substitution pattern of both phenyl rings (**7ab**, **7ac**, **7ad**) raised the metabolic stability in RLMs in comparison to the unsubstituted (**7t**) and the amine derivative (**7ae**) (Table 5). For further selection, the solubility limit in DPBS (Dulbecco's phosphate-buffered saline) buffer was determined. Therefore, the absorption change of a dilution series of the tested compound in buffer was measured and compared to buffer control. The solubility can be considered as moderate, and most modifications did not influence the solubility; just **7ac** shows a slightly higher solubility (Table 5). Additionally, we determined the toxicity

Table 4. Structure–Activity Relationship of the Introduced Moieties Raising the Metabolic Stability



No.	R_3	Inhibition sEH IC_{50} [μM][d]	Inhibition 5-LOX IC_{50} [μM][e]
7u[a]		0.002 (0.001-0.002)	0.16 (0.12-0.22)
7v[a]		0.0027 (0.0025-0.0032)	0.15 (0.12-0.19)
7w[a]		0.007 (0.006-0.007)	0.11 (0.08-0.15)
7x[a]		0.002 (0.002-0.003)	0.12 (0.09-0.17)
7y[b]		0.003 (0.002-0.003)	0.9 (0.6-1.2)
7z[b]		0.0024 (0.0022-0.0027)	1.0 (0.7-1.4)
7aa[b]		0.0021 (0.0020-0.0022)	0.096 (0.076 – 0.122)
7ab[b]		0.0016 (0.0016-0.0017)	0.22 (0.16-0.32)
7ac[b]		0.0024 (0.0021-0.0028)	0.26 (0.21-0.33)
7ad[c]		0.0012 (0.0010-0.0015)	0.11 (0.08-0.15)
7ae[c]		0.005 (0.004-0.006)	0.11 (0.08-0.15)
7af[b]		0.0030 (0.0029-0.0032)	0.16 (0.11-0.22)
7ag[b]		0.0030 (0.0029-0.0033)	0.23 (0.14-0.39)

[a]Diastereomeric ratio undetermined. [b]*R* (stereoisomer near the *N*-hydroxyurea moiety), diastereomeric ratio undetermined (stereoisomer next to the benzhydryl moiety). [c]*R* configuration. [d]All values were measured at least thrice in triplicate ($n \geq 3$). A 95% confidence interval is displayed in brackets. [e]All values were measured at least thrice ($n \geq 3$). A 95% confidence interval is displayed in brackets.

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of selected compounds with a CellTiter-Glo luminescent cell viability assay kit. Luciferase catalyzes with the help of ATP Beetle Luciferin to oxyluciferin, and the luminescence is detected using a luminescence reader. The number of viable cells is determined by quantification of ATP, which is an indicator of active cells. Compound **7ad** showed no cell toxicity up to a concentration of 25 μM; in contrast, **7ab** exhibited cell toxicity already at 7 μM (Figure S1). Hence, **7ad** was selected for detailed analysis.

Cellular and In Vivo Characterization. The efficacy concerning 5-LOX was demonstrated on a cellular level. Therefore, **7ad** was tested in polymorphonuclear leukocytes (PMNLs), where the formation of LOX products 5-HETE and LTB_4 was monitored by HPLC. **7ad** could enter the cell and inhibited selectively 5-LOX (Figure 5A, IC_{50} = 0.21 μM) and slightly enhanced the activity of 12- and 15-LOX (data not shown). The inhibitory potency in PMNLs was comparable to the reference inhibitor **5**.

Encouraged by the previous results, we performed an in vivo PK/PD study of **7ad** in male Swiss CD-1 mice. Therefore, an oral single dose of 3 mg/kg was administered to nine mice and three additional mice got the vehicle (1% methylcellulose/Tween80 99:1) as the control. The isolated serum was analyzed with LC−MS and showed that **7ad** has an acceptable pharmacokinetic profile (Figure 5B, C_{max} = 0.7 μM, t_{max} = 1 h, $t_{1/2}$ = 1.3 h). Effective concentrations above the IC_{50} values of sEH and 5-LOX could be reached over 3−4 h. Furthermore, we analyzed the EET/DHET ratio to verify the sEH inhibition. If the sEH is inhibited, then the EETs should accumulate and the ratio of EET/DHET is increased. This effect could be observed in the plasma samples of the treated mice (Figure 5C). The evaluation of 5-LOX target engagement in vivo was not possible in naïve mice that were not treated with an inflammatory stimulus. Hence, **7ad** seems to be suitable for inhibiting sEH and 5-LOX in vivo.

To investigate the therapeutic potential of **7ad**, the compound was applied intraperitoneally into mice subjected to unilateral ureter obstruction (UUO) to trigger kidney inflammation and fibrosis. Treatment with **7ad** strongly diminished UUO-induced tubulointerstitial fibrosis, as demonstrated by attenuated blue staining of collagen fibers in AZAN-stained sections (Figure 6A) and reduced fibrillar collagen in Sirius Red-stained sections (Figure 6B,C,E). Furthermore, the attenuated fibrotic response upon **7ad** application was confirmed by diminished mRNA expression of classic fibrogenic markers, such as collagen I (Col1a1) and fibronectin-1 (FN1) (Figure 6G). The infiltration of macrophages into tubulointerstitial regions correlates with inflammation and fibrosis severity and was therefore assessed using an F4/80 antibody.[35] **7ad** significantly ameliorated UUO-induced macrophage infiltration into the obstructed kidneys (Figure 6D,F) and attenuated the mRNA synthesis of Ccl2 (also known as MCP1), an important chemoattractant for macrophages, and of pro-inflammatory TNFα (Figure 6H). These data suggest the use of **7ad** as a promising therapeutic approach to treat inflammatory and fibrotic conditions.

Table 5. Microsomal Stability and Water Solubility of Selected Inhibitors

compound	% remaining compound after 60 min[a]	solubility limit in DPBS buffer[b]
7t	27 ± 6	between 3 and 10 μM
7ab	66 ± 2	between 3 and 10 μM
7ac	43 ± 14	between 10 and 30 μM
7ad	50 ± 2	between 3 and 10 μM
7ae	17 ± 4	between 3 and 10 μM
5	47 ± 2	between 30 and 100 μM

[a] $n \geq 3$. [b] $n = 2$, each measurement was in triplicate.

CONCLUSIONS

In this study, we were able to design and synthesize an orally available potent dual sEH/5-LOX inhibitor. Compound **7ad** was optimized by subsequent variation of the lipophilic parts of the initially designed prototype inhibitor **7a** considering in vitro inhibitory potency toward both enzymes and in vitro metabolic stability. The SAR analysis was strengthened by the X-ray structure of sEH in complex with **7ad**. Compound **7ad** can be used as a tool to investigate the therapeutic potential of dual sEH/5-LOX inhibitors in vivo using rodent models of diseases related to acute and chronic inflammation. Furthermore, physiological and pathophysiological consequences of

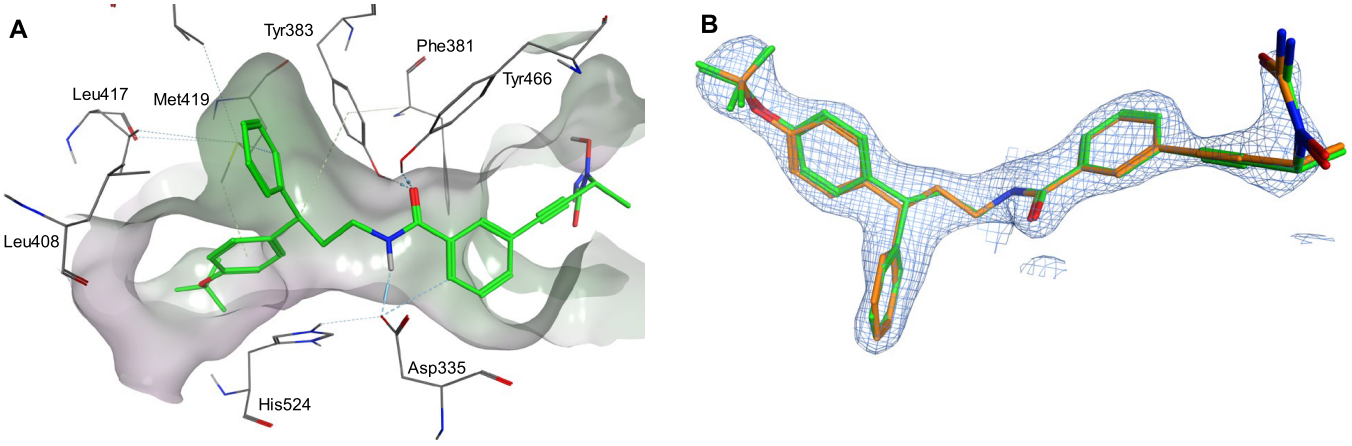

Figure 4. Analysis of the binding modes of **7w** with sEH. (A) X-ray structure of **7w** in complex with the C-terminal domain of sEH (PDB code 6YL4). Only one possible diastereomer of **7w** is displayed for clarity. (B) $2F_o-F_c$ electron density map at 1σ (blue mesh) of both diastereomers (green and orange sticks) of **7w**.

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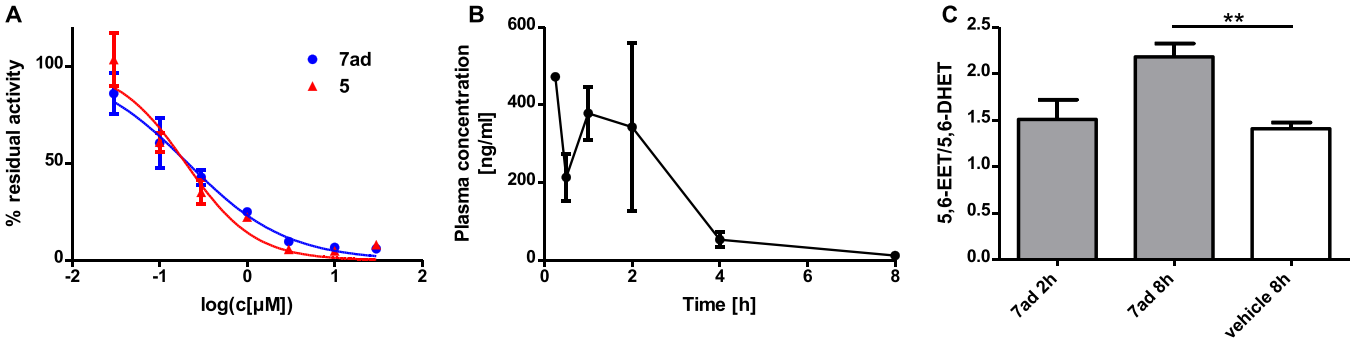

Figure 5. Evaluation of **7ad** in human PMNL and in mice. (A) 5-LOX activity in human PMNLs. (B) Pharmacokinetic profiling of **7ad** in male Swiss CD-1 mice (3 mg/kg dosage, p.o., three mice per time point). **7ad** revealed an acceptable bioavailability and a half-life of 1.3 h. (C) Plasma samples of the naïve mice were analyzed regarding their sEH substrate/metabolized substrate (EETs/DHETs) ratio. Statistical analysis was performed with the Bonferroni correction: ** = 0.005.

dual inhibition of the CYP and LOX branch of the AA cascade on the lipidome level can be accessed upon application of **7ad**.

EXPERIMENTAL SECTION

Chemistry Materials and General Procedures. All purchased solvents and chemicals from different suppliers such as Sigma Aldrich Chemie GmbH, Acros Organics, Alfa Aesar, Fluorochem, and TCI Europe were used without further purification. Reactions, carried out with microwave irradiation, were performed in a device Biotage Initiator 2.0 from Biotage. For thin-layer chromatography (TLC), TLC plates F254 from Merck were used, and aromatic systems were visualized with ultraviolet light (254 and 365 nm). Non-aromatic systems were visualized with a ninhydrin ethanol solution. Purification with column chromatography occurred either with silica gel 60 (230−400 mesh, ASTM) from Fluka or with a flash system from in Puriflash XS420 from Interchim with columns from Biotage (particle size of 50 μm) or Agela Technologies (particle size of 60 Å). NMR spectra were recorded on a DPX250, an AV300, an AV400, and an AV500. The chemical shifts were reported in ppm relative to tetramethylsilane. Spectra were calibrated with the internal signal of the non-deuterated solvent. The reported multiplicities are singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet (q), and multiplet (m), and coupling constant (*J*) are given in hertz (Hz). The purity of the compounds was determined by HPLC (LCMS 2020 from Shimadzu). The columns Luna 10 μ C18(2) 100A (250 × 4.60 mm) and Luna 10 μ C18(2) (250 × 21.20 mm) from Phenomenex were used for analytical and preparative purposes, respectively. UPLC runs were performed with a MultoHigh UC (50 mm × 2 mm) column from CS Chromatography-Service GmbH. Conditions were as follows: eluent, ACN/0.1% aqueous formic acid, flow rate was 0.5 mL/min (UPLC), 1 mL/min (scout column), or 21 mL/min (semi-preparative column) with UV monitoring at 254 and 280 nm. The specific conditions were described in the experimental procedures of the compounds. **7ag** was isolated with a chiral column Chiralcel OJ-RH (4.6 × 150 mm, particle size 5 μm) from Daicel. The eluent of the used HPLC System Agilent 1290 infinity II was ACN and 0.1% aqueous formic acid with a flow rate of 0.5 mL/min. The runs were isocratic with 36% ACN. All final compounds exhibit a purity over 95% at 254 nm. Mass detection occurred either on an LCMS-2020 from Shimadzu or a VG platform II from Fison instruments Ltd. High-resolution mass was measured in a MALDI LTQ Orbitrap XL instrument from Thermo Scientific.

If the used equivalents differ from the common procedure, the equivalents are listed in the respective experiment.

Phenyl (phenoxycarbonyl)-oxycarbamate (**12**) was synthesized according to the published literature.[36]

General Procedure for the Synthesis of Protected *N*-Hydroxy Carbamates 14a−h (Procedure A). Under an inert atmosphere, 1.1 equiv of PPh_3, 0.9 equiv of alkyne derivatives **13a−h**, and 1 equiv of **12** were dissolved in dry THF and cooled to 0 °C. To this solution, 1.1 equiv of DIAD was added dropwise while waiting for the decolorization of DIAD between each drop. The solution was stirred for 1 h at room temperature. The solvent was evaporated under reduced pressure, and the yellow oil was pre-adsorbed on silica gel and purified with column chromatography. An oil was obtained.

Phenyl But-3-yn-2-yl((phenoxycarbonyl)oxy)carbamate ***14a.*** Procedure A; 1.67 g (6.36 mmol) PPh_3, 1.3 mL (6.36 mmol) DIAD, 0.5 mL (5.20 mmol) **13a** (3-butyn-2-ol), 1.58 g (5.78 mmol) **12**, 25 mL THF; eluent of column chromatography hexane/Et_2O 3:1−2:1; yield: yellow oil (1.46 g, 4.49 mmol, 78%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 7.54−7.20 (m, 10H), 5.39−5.29 (m, 1H), 3.62 (d, *J* = 1.6 Hz, 1H), 1.55 (d, *J* = 6.9 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 150.4, 130.0, 129.8, 127.0, 126.6, 121.4, 120.7, 115.2, 30.6 ppm.

Phenyl (Phenoxycarbonyl)oxy(prop-2-yn-1-yl)carbamate ***14b.*** Procedure A; 0.4 mL (6.65 mmol) **13b** (2-propyn-1-ol), 1.8 mL (8.87 mmol) DIAD, 2.33 g (8.87 mmol) PPh_3, 2.02 g (7.39 mmol) **12**, 25 mL THF; eluent of column chromatography hexane/Et_2O 3:1−2:1; yield: transparent oil (2.24 g, 7.20 mmol, 98%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 7.53−7.43 (m, 4H), 7.40−7.32 (m, 4H), 7.24−7.17 (m, 2H), 4.76 (d, *J* = 2.4 Hz, 2H), 3.53 (t, *J* = 2.4 Hz, 1H) ppm; MS (ESI) *m/z*: 312.00 [M + H$^+$].

(S)-Phenyl But-3-yn-2-yl((phenoxycarbonyl)oxy)carbamate ***14c.*** Procedure A; 0.5 mL (6.65 mmol) **13c** ((*R*)-3-butyn-2-ol,) 1.7 mL (8.87 mmol) DIAD, 2.33 g (8.87 mmol) PPh_3, 2.02 g (7.39 mmol) **12**, 20 mL THF; eluent of column chromatography hexane/Et_2O 3:1; yield: white oil (1.99 g, 6.12 mmol, 83%); ^{1}H-NMR (250 MHz, $CDCl_3$) δ = 7.59−7.42 (m, 4H), 7.42−7.27 (m, 4H), 7.24−7.19 (m, 2H), 5.33 (qd, *J* = 2.3, 6.9 Hz, 1H), 3.61 (d, *J* = 1.1 Hz, 1H), 1.56 (d, *J* = 6.9 Hz, 3H) ppm; MS (ESI) *m/z*: 325.95 [M + H$^+$].

(R)-Phenyl But-3-yn-2-yl((phenoxycarbonyl)oxy)carbamate ***14d.*** Procedure A; 0.5 mL (6.65 mmol) **13d** ((*S*)-3-butyn-2-ol), 1.7 mL (8.87 mmol) DIAD, 2.33 g (8.87 mmol) PPh_3, 2.02 g (7.39 mmol) **12**, 20 mL THF; eluent of column chromatography hexane: acetone 1:1; yield: white oil (0.47 g, 1.45 mmol, 20%); ^{1}H-NMR (250 MHz, $CDCl_3$) δ = 7.51−7.34 (m, 4H), 7.33−7.10 (m, 6H), 5.30−5.20 (m, 1H), 2.46−2.44 (m, 1H), 1.56 (d, *J* = 7.0 Hz, 3H) ppm; MS (ESI) *m/z*: 325.85 [M + H$^+$].

Phenyl Pent-1-yn-3-yl((phenoxycarbonyl)oxy)carbamate ***14e.*** Procedure A; 0.1 mL (1.65 mmol) **13e** (1-pentyn-3-ol), 0.4 mL (2.20 mmol) DIAD, 0.58 g (2.20 mmol) PPh_3, 0.50 g (1.83 mmol) **12**, 11 mL THF; eluent of column chromatography hexane/Et_2O 3:1−2:1; yield: yellow oil (0.43 g, 1.26 mmol, 69%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 7.54−7.43 (m, 4H), 7.40−7.29 (m, 4H), 7.24−7.19 (m, 2H), 5.13−5.09 (m, 1H), 3.62 (d, *J* = 2.1 Hz, 1H), 1.97−1.85 (m, 2H), 1.05 (t, *J* = 7.3 Hz, 3H) ppm; MS (ESI) *m/z*: 339.90 [M + H$^+$].

Phenyl (4-Methylpent-1-yn-3-yl)((phenoxycarbonyl)oxy)-carbamate ***14g.*** Procedure A; 0.7 mL (6.65 mmol) **13g** (4-methyl-1-pentyn-3-ol), 1.8 mL (8.87 mmol) DIAD, 2.33 g (8.87 mmol) PPh_3, 2.02 g (7.39 mmol) **12**, 25 mL THF; eluent of column chromatography hexane/Et_2O 3:1−2:1; yield: yellow oil (1.35 g, 3.84 mmol, 52%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 7.57−7.41

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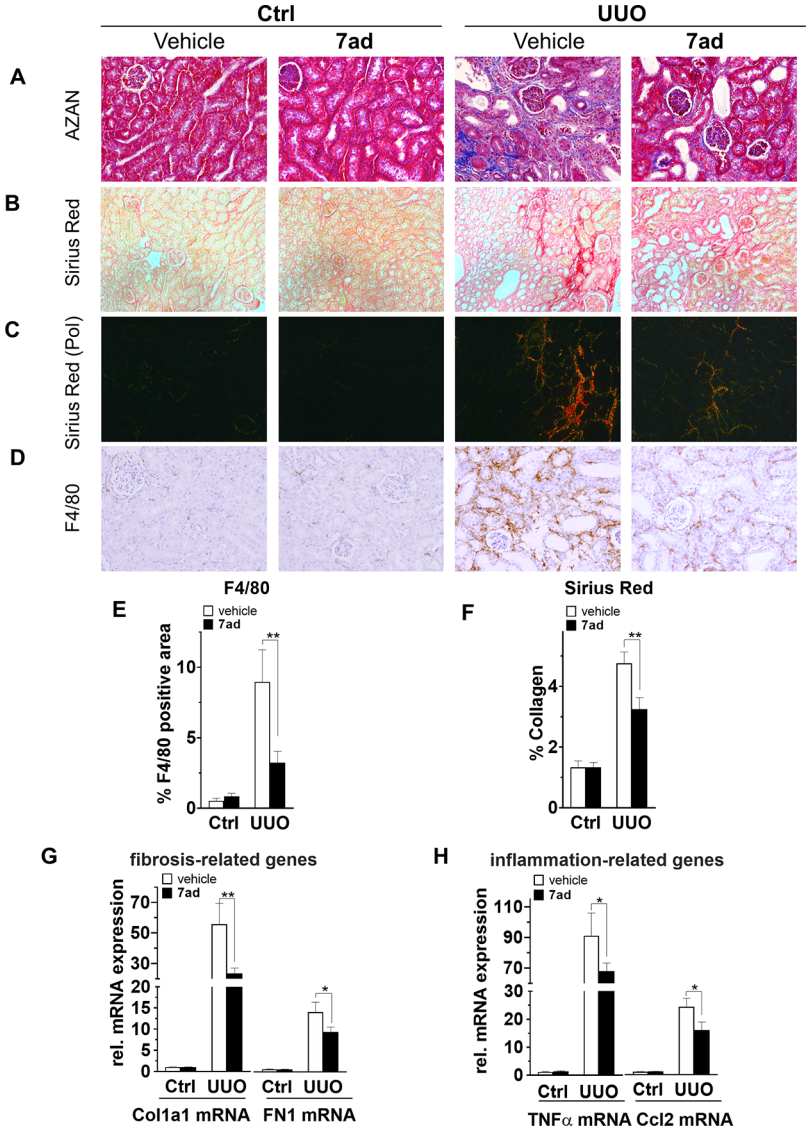

Figure 6. 7ad exhibits anti-inflammatory and anti-fibrotic activities in the UUO model. (A) AZAN, (B, C) Sirius Red, and (D) F4/80 staining demonstrating fibrotic changes and macrophage infiltration in representative kidney sections of ligated (UUO) and contralateral kidneys of vehicle- or **7ad**-treated mice after 7 days. (B, C) Sirius Red-stained sections were imaged under bright light (Sirius Red) and under polarized light (Sirius Red (Pol)) to detect birefringence of collagen fibers. Graphs show (E) the collagen quantification of Sirius Red or (F) macrophage-positive area of F4/80-stained kidney sections of vehicle- (white bars) or **7ad**-treated (black bars) mice using ImageJ software version 1.51k. (G) Real-time quantitative RT-PCR analysis normalized to GAPDH of fibrosis-associated genes Col1a1 and FN1 and (H) inflammation-associated genes TNFα and Ccl2 of whole kidney homogenates of ligated (UUO) and contralateral (Ctrl) kidneys of vehicle- (white bars) or **7ad**-treated (black bars) mice at day 7. Data are expressed as means ± SEM and $n = 5–6$ in (E) and (F). *$p < 0.05$ and **$p < 0.01$ compared to UUO kidneys of vehicle-treated mice using two-way analysis of variance and a Sidak post hoc test.

(m, 5H), 7.41–7.25 (m, 3H), 7.22–7.17 (m, 2H), 4.91–4.89 (m, 1H), 3.65 (d, J = 1.9 Hz, 1H), 2.24–2.17 (m, 1H), 1.08 (d, J = 7.0 Hz, 6H) ppm; MS (ESI) m/z: 353.85 [M + H^+].

Phenyl (5-Methylhex-1-yn-3-yl)((phenoxycarbonyl)oxy)-carbamate ***14h***. Procedure A; 0.9 mL (6.65 mmol) **13h** (5-methyl-1-hexyn-3-ol), 1.7 mL (8.87 mmol) DIAD, 2.33 g (8.87 mmol) PPh_3, 2.02 g (7.39 mmol) **12**, 25 mL THF; eluent of column chromatography hexane/Et_2O 4:1–2:1; yield: transparent oil (1.62 g, 4.41 mmol, 60%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 7.57–7.26 (m, 8H), 7.26–7.19 (m, 2H), 5.17–5.09 (m, 1H), 3.64 (d, J = 2.2 Hz, 1H), 1.79 (d, J = 9.7 Hz, 3H), 1.01–0.91 (m, 6H) ppm; MS (ESI) m/z: 367.90 [M + H^+].

General Procedure for the Synthesis of Deprotected *N*-Hydroxy Ureas 8a–h (Procedure B). **14a–h** were dissolved and cooled with liquid nitrogen. Ammonia was condensed to this mixture, and the solution was transferred to an autoclave. The reaction was stirred under an ammonia atmosphere (5–7 bar) for 16–20 h at room temperature. After the reaction time, the autoclave was ventilated and stirred for 1 h under ambient conditions. The solvent was removed under reduced pressure, and the residue was purified via column chromatography.

1-(But-3-yn-2-yl)-1-hydroxyurea ***8a***. Procedure B; 0.38 g (1.17 mmol) **14a**, 3 bar NH_3 atmosphere, 4 mL *tert*-butyl alcohol, eluent of column chromatography DCM/MeOH 9:1, dirty white solid (0.91 g, 0.71 mmol, 61%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.22 (s, 1H), 6.47 (s, 2H), 4.85 (qd, J = 2.3, 7.0 Hz, 1H), 3.03 (d, J = 2.3 Hz, 1H), 1.25 (d, J = 7.1 Hz, 3H) ppm; MS (ESI) m/z: 151.05 [M + Na^+].

1-Hydroxy-1-(prop-2-yn-1-yl)urea ***8b***. Procedure B; 1.17 g (3.75 mmol) **14b**, 5 bar NH_3 atmosphere, 12 mL *tert*-butyl alcohol/cyclohexane 1:1, eluent of column chromatography DCM/MeOH 9:1, brown solid (0.41 g, 3.58 mmol, 96%); ^{1}H-NMR (250 MHz,

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DMSO-d_6) δ = 9.48 (s, 1H), 6.48 (s, 2H), 4.05 (d, J = 2.4 Hz, 2H), 3.05 (t, J = 2.4 Hz, 1H) ppm; MS (ESI) m/z: 114.90 [M + H$^+$].

(S)-1-(But-3-yn-2-yl)-1-hydroxyurea ***8c***. Procedure B; 2.75 g (8.44 mmol) **14c**, 4.5 bar NH_3 atmosphere, 6 mL *tert*-butyl alcohol/THF 1:3, eluent of column chromatography DCM/MeOH 95:5−9:1, yellow solid (0.55 g, 4.32 mmol, 51%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.23 (s, 1H), 6.49 (s, 2H), 4.86 (qd, J = 2.3, 7.0 Hz, 1H), 3.04 (d, J = 2.3 Hz, 1H), 1.25 (d, J = 7.1 Hz, 3H) ppm; MS (ESI) m/z: 129.00 [M + H$^+$].

(R)-1-(But-3-yn-2-yl)-1-hydroxyurea ***8d***. Procedure B; 1.71 g (5.26 mmol) **14d**, 7 bar NH_3 atmosphere, 10 mL isopropyl alcohol, eluent of column chromatography DCM/MeOH$_{ammonia}$ 98:1−9:1, dirty white solid (0.56 g, 4.35 mmol, 83%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.22 (s, 1H), 6.47 (s, 2H), 4.85 (qd, J = 2.3, 7.0 Hz, 1H), 3.03 (d, J = 2.3 Hz, 1H), 1.25 (d, J = 7.0 Hz, 3H) ppm; MS (ESI) m/z: 129.05 [M + H$^+$].

1-Hydroxy-1-(pent-1-yn-3-yl)urea ***8e***. Procedure B; 0.42 g (1.24 mmol) **14e**, 6 bar NH_3 atmosphere, 6 mL THF/hexane 3:1, eluent of column chromatography DCM/MeOH 98:2−9:1, white solid (0.14 g, 0.96 mmol, 77%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.18 (s, 1H), 6.46 (s, 2H), 4.62 (td, J = 7.8, 2.3 Hz, 1H), 3.05 (d, J = 2.3 Hz, 1H), 1.70−1.58 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H) ppm; MS (ESI) m/z: 143.05 [M + H$^+$].

1-(Hex-1-yn-3-yl)-1-hydroxyurea ***8f***. Procedure B; 0.65 g (1.83 mmol) **14f**, 6 bar NH_3 atmosphere, 6 mL THF/hexane 3:1, eluent of column chromatography DCM/MeOH 98:2−9:1, white solid (0.15 g, 0.96 mmol, 52%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.17 (s, 1H), 6.45 (s, 2H), 4.72 (td, J = 7.7, 2.3 Hz, 1H), 3.03 (d, J = 2.3 Hz, 1H), 1.78−1.16 (m, 4H), 0.87 (t, J = 7.3 Hz, 3H) ppm; MS (ESI) m/z: 156.60 [M + H$^+$].

1-Hydroxy-1-(4-methylpent-1-yn-3-yl)urea ***8g***. Procedure B; 1.33 g (3.75 mmol) **14g**, 7 bar NH_3 atmosphere, 12 mL *tert*-butyl alcohol/hexane 1:1, eluent of column chromatography DCM/MeOH 100:0−9:1, dirty white solid (0.53 g, 3.37 mmol, 90%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.14 (s, 1H), 6.44 (s, 2H), 4.36 (dd, J = 2.3, 9.9 Hz, 1H), 3.05 (d, J = 2.3 Hz, 1H), 1.99−1.82 (m, 1H), 1.00 (d, J = 6.8 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H) ppm; MS (ESI) m/z: 156.90 [M + H$^+$].

1-Hydroxy-1-(5-methylhex-1-yn-3-yl)urea ***8h***. Procedure B; 1.60 g (4.35 mmol) **14h**, 4 bar NH_3 atmosphere, 12 mL *tert*-butyl alcohol/hexane 1:1, eluent of column chromatography DCM/MeOH 100:0−9:1, dirty yellow solid (0.53 g, 3.08 mmol, 71%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.17 (s, 1H), 6.46 (s, 2H), 4.79 (dt, J = 2.3, 7.8 Hz, 1H), 3.03 (d, J = 2.3 Hz, 1H), 1.87−1.35 (m, 3H), 0.87 (dd, J = 3.7, 6.8 Hz, 6H) ppm; MS (ESI) m/z: 170.90 [M + H$^+$].

General Procedure for the Synthesis of Amide Derivatives 15a−j from Acyl Chlorides 16a,b (Procedure C). 4-Iodobenzoyl chloride **16a** or 3-iodobenzoyl chloride **16b** (1 equiv) was dissolved in dry 1,2-dichloroethane. At 0 °C, 1 equiv of the corresponding amine **10a−10i** and 3 equiv of DIPEA were added and heated under microwave irradiation in a sealed tube to 90 °C. After cooling to room temperature, the solvent was removed, and the residue was resolved in ethyl acetate. The organic phase was washed with water (3×), saturated aqueous $NaHCO_3$ solution (2×), 2 M aqueous HCl (2×), and brine (1×). After drying over $MgSO_4$ and filtration, the organic solvent was evaporated under reduced pressure. The solid was purified with column chromatography.

4-Iodo-N-(2-(trifluoromethyl)benzyl)benzamide ***15a***. Procedure C; 0.63 g (2.36 mmol) **16a**, 0.3 mL (2.36 mmol) **10a**, 0.8 mL (4.37 mmol) DIPEA, 3 mL DCE; white solid (0.81 g, 2.00 mmol, 84%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.17 (t, J = 5.7 Hz, 1H), 7.92−7.87 (m, 2H), 7.75−7.62 (m, 4H), 7.53−7.45 (m, 2H), 4.65 (d, J = 5.8 Hz, 2H) ppm; MS (ESI) m/z: 405.80 [M + H$^+$].

3-Iodo-N-(2-(trifluoromethyl)benzyl)benzamide ***15b***. Procedure C; 0.33 g (1.24 mmol, 0.7 equiv) **16b**, 0.2 mL (1.81 mmol) **10a**, 0.6 mL (3.72 mmol, 3.0 equiv) DIPEA, 5 mL DCE; white solid (0.44 g, 1.10 mmol, 88%), ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.19 (t, J = 5.7 Hz, 1H), 8.28 (t, J = 1.6 Hz, 1H), 7.93 (dd, J = 1.7, 7.8 Hz, 2H), 7.81−7.59 (m, 2H), 7.59−7.41 (m, 2H), 7.31 (t, J = 7.8 Hz, 1H), 4.65 (d, J = 5.6 Hz, 2H) ppm; MS (ESI) m/z: 405.70 [M + H$^+$].

N-(2-(1H-Indol-3-yl)ethyl)-3-iodobenzamide ***15c***. Procedure C; 0.29 g (1.09 mmol) **16b**, 0.38 g (2.40 mmol, 2.2 equiv) **10b** (2-(1*H*-indol-3-yl)ethanamine), 1.1 mL (6.54 mmol) DIPEA, 5 mL DCE; eluent of column chromatography hexane/ethyl acetate 1:3, brownish solid (0.33 g, 0.84 mmol, 77%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 10.82 (s, 1H), 8.69 (t, J = 5.6 Hz, 1H), 8.18 (t, J = 1.6 Hz, 1H), 7.94−7.72 (m, 2H), 7.60−7.54 (m, 1H), 7.40−6.92 (m, 5H), 3.59−3.47 (m, 2H), 2.94 (t, J = 7.4 Hz, 2H) ppm; MS (ESI) m/z: 389.01 [M − H$^+$].

N-(4-Fluorophenethyl)-3-iodobenzamide ***15d***. Procedure C; 0.48 g (1.81 mmol, 1.6 equiv) **16b**, 0.1 mL (1.13 mmol) **10c** (3-fluorophenethylamine), 0.6 mL (3.39 mmol, 3.0 equiv) DIPEA, 5 mL DCE; eluent of column chromatography hexane/acetone 2:1; yellow oil (0.40 g, 1.09 mmol, 96%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 8.62 (t, J = 5.4 Hz, 1H), 8.14 (t, J = 1.6 Hz, 1H), 7.93−7.73 (m, 2H), 7.34−7.19 (m, 3H), 7.15−7.06 (m, 2H), 3.50−3.40 (m, 2H), 2.82 (t, J = 7.3 Hz, 2H) ppm; MS (ESI) m/z: 369.80 [M + H$^+$].

N-(3-Fluorobenzyl)-3-iodobenzamide ***15e***. Procedure C; 0.48 g (1.81 mmol, 1.6 equiv) **16b**, 0.1 mL (1.13 mmol) **10d** (3-fluorobenzylamine), 0.6 mL (3.39 mmol, 3.0 equiv) DIPEA, 5 mL DCE; eluent of column chromatography hexane/acetone 2:1; yellow oil (0.38 g, 1.08 mmol, 95%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.15 (t, J = 5.7 Hz, 1H), 8.24 (t, J = 1.6 Hz, 1H), 7.98−7.83 (m, 2H), 7.44−7.23 (m, 2H), 7.22−6.94 (m, 3H), 4.47 (d, J = 5.9 Hz, 2H) ppm; MS (ESI) m/z: 353.80 [M − H$^+$].

N-(2,4-Dichlorobenzyl)-3-iodobenzamide ***15f***. Procedure C; 0.53 g (1.99 mmol, 1.6 equiv) **16b**, 0.2 mL (1.24 mmol) **10e** (2,4-dichlorophenyl)methanamine), 0.6 mL (3.72 mmol, 3.0 equiv) DIPEA, 5 mL DCE; white solid (0.40 g, 1.20 mmol, 97%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.15 (t, J = 5.4 Hz, 1H), 8.25 (t, 1.6 Hz, 1H), 8.06−7.82 (m, 2H), 7.62 (d, J = 1.8 Hz, 1H), 7.58−7.25 (m, 3H), 4.50 (d, J = 5.6 Hz, 2H) ppm; MS (ESI) m/z: 405.70 [M + H$^+$].

3-Iodo-N-(4-methoxy-2-(trifluoromethyl)benzyl)benzamide ***15g***. Procedure C; 0.48 g (1.81 mmol, 1.5 equiv) **16b**, 0.25 g (1.24 mmol) **10f** (4-methoxy-2-(trifluoromethyl)phenyl)methanamine), 0.6 mL (3.72 mmol, 3.0 equiv) DIPEA, 5 mL DCE; white solid (0.46 g, 1.05 mmol, 85%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.10 (t, J = 5.6 Hz, 1H), 8.25 (t, J = 1.6 Hz, 1H), 7.94−7.88 (m, 2H), 7.48−7.42 (m, 1H), 7.34−7.20 (m, 3H), 4.56 (d, J = 5.5 Hz, 2H), 3.81 (s, 3H) ppm; MS (ESI) m/z: 435.80 [M + H$^+$].

N-(4-Fluoro-2-(trifluoromethyl)benzyl)-3-iodobenzamide ***15h***. Procedure C; 0.48 g (1.81 mmol) **16b**, 0.3 mL (1.37 mmol) **10g** (4-fluoro-2-(trifluoromethyl)-phenyl)methanamine), 0.7 mL (4.11 mmol, 3.0 equiv) DIPEA, 5 mL DCE; eluent of column chromatography hexane/acetone 2:1; yellow oil (0.51 g, 1.21 mmol, 89%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.20 (t, J = 5.3 Hz, 1H), 8.28−8.24 (m, 1H), 7.97−7.89 (m, 2H), 7.67−7.50 (m, 3H), 7.31 (t, J = 7.8 Hz, 1H), 4.61 (d, J = 5.3 Hz, 2H) ppm; MS (ESI) m/z: 423.75 [M + H$^+$].

N-(3,3-Diphenylpropyl)-3-iodobenzamide ***15i***. Procedure C; 0.33 g (1.24 mmol) **16b**, 0.26 g (1.24 mmol) **10h** (3,3-diphenylpropan-1-amine), 0.6 mL (3.72 mmol, 3.0 equiv) DIPEA, 5 mL DCE; white solid (0.42 g, 0.94 mmol, 76%), ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 8.55 (t, J = 5.2 Hz, 1H), 8.15 (t, J = 1.6 Hz, 1H), 7.90−7.79 (m, 2H), 7.31−7.25 (m, 11H), 4.08−3.99 (m, 1H), 3.22−3.10 (m, 2H), 2.30 (q, J = 7.5 Hz, 2H) ppm; MS (ESI) m/z: 441.80 [M + H$^+$].

N-(3-(4-Fluorophenyl)-3-phenylpropyl)-3-iodobenzamide ***15j***. Procedure C; 0.18 g (0.69 mmol) **16b**, 0.16 g (0.69 mmol) **10i**, 0.4 mL (2.05 mmol) DIPEA, 3 mL DCE, eluent of column chromatography hexane/ethyl acetate 3:1; white solid (0.13 g, 0.28 mmol, 41%), ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 8.55 (t, J = 5.2 Hz, 1H), 8.15 (t, J = 1.6 Hz, 1H), 7.90−7.79 (m, 2H), 7.31−7.25 (m, 10H), 4.08−3.99 (m, 1H), 3.22−3.10 (m, 2H), 2.30 (q, J = 7.5 Hz, 2H) ppm; MS (ESI) m/z: 460.00 [M + H$^+$].

3-Iodo-N-(2-(trifluoromethoxy)benzyl)benzamide ***15k***. To a solution of 190 mg (0.71 mmol) of **16b** in 5 mL DCM 0.4 mL of (2.14 mmol, 3 equiv) **10j** (2-(trifluoromethoxy)phenyl)-methanamine) and 2 mg of (0.01 mmol, 0.02 equiv) 4-DMAP were added. Additionally, 0.4 mL of (2.14 mmol, 3 equiv) DIPEA was added and the mixture stirred for 18 h at room temperature. The

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organic phase was washed with water (2×), 2 M aqueous HCl (2×), and brine (1×). After drying over $MgSO_4$ and filtration, the organic solvent was evaporated under reduced pressure. A brown solid was obtained (0.29 g, 0.68 mmol, 95%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.11 (t, J = 5.7 Hz, 1H), 8.25 (t, J = 1.6 Hz, 1H), 7.92 (td, J = 1.5, 7.8 Hz, 2H), 7.69−7.19 (m, 5H), 4.53 (d, J = 5.8 Hz, 2H) ppm; MS (ESI) m/z: 421.75 [M + H$^+$].

General Procedure for the Synthesis of Amide Derivatives 15l−q (Procedure D). Acid derivative **17a** or **17b** (1 equiv) was dissolved in dry THF, and 1.8 equiv of PyBOP was added. The corresponding amine **10** (1.1 equiv) and 2 equiv of DIPEA or DIPA, dissolved in dry THF, were added. The reaction was stirred for 16−20 h at room temperature, and afterward the solvent was removed. The organic phase was washed with water (3×), saturated aqueous $NaHCO_3$ solution (2×), and brine (1×). After drying over $MgSO_4$ and filtration, the organic solvent was evaporated under reduced pressure. The solid was purified with column chromatography.

3-Iodo-4-methyl-N-(2-(trifluoromethyl)benzyl)benzamide ***15l***. Procedure D; **10a**, 0.15 g (0.57 mmol), 0.53 g (1.02 mmol) PyBOP, 0.2 mL (1.14 mmol) DIPA in 15 mL THF; white solid (0.19 g, 0.45 mmol, 79%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.13 (t, J = 5.7 Hz, 1H), 8.37 (d, J = 1.8 Hz, 1H), 7.86 (dd, J = 1.8, 7.9 Hz, 1H), 7.80−7.58 (m, 2H), 7.58−7.39 (m, 3H), 4.64 (d, J = 5.5 Hz, 2H), 2.42 (s, 3H) ppm; MS (ESI) m/z: 419.75 [M + H$^+$].

3-Iodo-N-(3-phenyl-3-(4-(trifluoromethyl)phenyl)propyl)-benzamide ***15m***. Procedure D; 0.13 g (0.47 mmol) **10k**, 0.12 g (0.47 mmol) **17a**, 0.36 g (0.70 mmol) PyBOP, 0.1 mL (0.36 mmol) DIPA in 10 mL THF; eluent of column chromatography hexane/ethyl acetate 3:1; transparent oil (0.17 g, 0.33 mmol, 72%); ^{1}H-NMR (250 MHz, acetone-d$_6$) δ = 8.20−8.18 (m, 1H), 8.04−7.72 (m, 3H), 7.72−7.53 (m, 4H), 7.43−7.14 (m, 6H), 4.26 (t, J = 7.8 Hz, 1H), 3.41−3.34 (m, 2H), 2.52−2.41 (m, 2H) ppm; MS (ESI) m/z: 509.80 [M + H$^+$].

3-Iodo-N-(3-phenyl-3-(4-(trifluoromethoxy)phenyl)propyl)-benzamide ***15n***. Procedure D; 0.15 g (0.52 mmol) **10l**, 0.13 g (0.52 mmol) **17a**, 0.30 g (0.57 mmol) PyBOP, 0.3 mL (1.55 mmol) DIPA in 13 mL THF; white solid (0.25 g, 0.47 mmol, 90%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 8.19 (t, J = 1.7 Hz, 1H), 7.90−7.83 (m, 3H), 7.52−7.45 (m, 2H), 7.40−7.18 (m, 8H), 4.21 (t, J = 7.8 Hz, 1H), 3.42−3.32 (m, 2H), 2.49−2.38 (m, 2H) ppm; MS (ESI) m/z: 525.85 [M + H$^+$].

N-(3-(4-Chlorophenyl)-3-phenylpropyl)-3-iodobenzamide ***15o***. Procedure D; 86 mg (350 μmol) **10m**, 86 mg (350 μmol) **17a**, 237 mg (525 μmol) PyBOP, 0.1 mL (700 μmol) DIPA, 17 mL THF, 2 mL DMF; eluent of flash chromatography hexane/ethyl acetate 3:1; white solid (112 mg, 235 μmol, 67%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 8.19 (t, J = 1.8 Hz, 1H), 7.89−7.83 (m, 3H), 7.41−7.15 (m, 10H), 4.15 (t, J = 8.1 Hz, 1H), 3.41−3.31 (m, 2H), 2.47−2.37 (m, 2H) ppm; MS (ESI) m/z: 475.85 [M + H$^+$].

N-(3-(3,4-Dichlorophenyl)-3-phenylpropyl)-3-iodobenzamide ***15p***. Procedure D; 0.50 g (1.78 mmol) **10n**, 0.45 g (1.78 mmol) **17a**, 1.02 g (1.96 mmol) PyBOP, 0.9 mL (5.34 mmol) DIPA, 10 mL THF, 1 h 60 °C microwave irradiation; eluent of flash chromatography hexane/acetone 4:1; yellow oil (0.86 g, 1.69 mmol, 95%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 8.18 (t, J = 2.5 Hz, 1H), 7.93−7.78 (m, 2H), 7.56 (d, J = 2.5 Hz, 1H), 7.50−7.46 (m, 1H), 7.44−7.13 (m, 8H), 4.19 (t, J = 7.5 Hz, 1H), 3.42−3.34 (m, 2H), 2.48−2.39 (m, 2H) ppm; MS (ESI) m/z: 511.05 [M + H$^+$].

N-(3-(4-Aminophenyl)-3-Phenylpropyl)-3-Iodobenzamide ***15q***. 0.23 g of (0.89 mmol, 1 equiv) **17a** and 0.46 g of (0.89 mmol, 1 equiv) PyBOP were suspended in dry THF, and 0.3 mL of (1.78 mmol, 2 equiv) DIPEA was added. The solution stirred for 13 h at room temperature. Afterward, it was added slowly to 0.20 g of (0.89 mmol) **10o** solved in 8 mL of dry THF. The mixture was stirred for 1 h at room temperature. The solvent was evaporated under reduced pressure, and the residue was dissolved in ethyl acetate. The organic phase was washed with saturated aqueous $NaHCO_3$ solution (2×), with saturated aqueous NH_4Cl solution (2×) and brine (1×). After drying over $MgSO_4$ and filtration, the organic solvent was removed under reduced pressure. The solid was purified with column chromatography (hexane/acetone 4:1−2:1), and a transparent oil was obtained (0.10 g, 0.23 mmol, 25%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 8.19 (t, J = 1.7 Hz, 1H), 7.88−7.77 (m, 3H), 7.31−7.22 (m, 6H), 7.04−6.99 (m, 2H), 6.62−6.57 (m, 2H), 4.44 (bs, 2H), 3.94 (t, J = 7.8 Hz, 1H), 3.39−3.31 (m, 2H), 2.37−2.29 (m, 2H) ppm; MS (ESI) m/z: 457.03 [M + H$^+$].

3-Iodo-N-(3-(4-(methylsulfonamido)phenyl)-3-phenylpropyl)-benzamide ***15r***. To a solution of 103 mg of (226 μmol, 1 equiv) **15q** in 10 mL dry DCM, 18 μL of (234 μmol, 1.04 equiv) methanesulfonyl chloride was added at 0 °C. 64 μL of (456 μmol, 2 equiv) NEt_3 was added, and the solution was stirred for 17 h under reflux conditions. The mixture was allowed to cool to room temperature, and 2 M aqueous HCl was added. The organic phase was separated, and the aqueous phase was extracted with ethyl acetate. The organic phases were combined, dried over $MgSO_4$, and filtered. After the removal of the solvent, the residue was purified with column chromatography (hexane/acetone 2:1). A transparent oil was obtained (108 mg, 202 μmol, 89%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 8.49 (bs, 1H), 8.21 (t, J = 1.7 Hz, 1H), 7.87 (dd, J = 1.7, 7.8 Hz, 2H), 7.37−7.21 (m, 11H), 4.11 (t, J = 7.8 Hz, 1H), 3.45−3.33 (m,2H), 2.93 (s, 3H), 2.47−2.36 (m, 2H). ppm; MS (ESI) m/z: 533.02 [M − H$^+$].

General Procedure for the Synthesis of Amide Derivatives 15s−y (Procedure E). **17a** (1 equiv), 1.1 equiv of PyBOP, 0.5 equiv of HOBt·H_2O, and 2 equiv of DIPEA were dissolved in abs THF, and 1 equiv of the corresponding amine **10**, dissolved in dry THF, was added portionwise. The mixture was either heated for 1 h via microwave irradiation at 60 °C or stirred 16−20 h at room temperature. The solvent was evaporated, and the residue was purified with column chromatography.

3-Iodo-N-(3-phenyl-3-(4-sulfamoylphenyl)propyl)benzamide ***15s***. Procedure E; 0.80 g (0.31 mmol) **17a**, 0.90 g (0.31 mmol) **10p**, 0.24 g (0.47 mmol) PyBOP, 0.07 g (0.47 mmol) HOBt·H_2O, 0.2 mL (0.93 mmol) DIPEA, 2 mL THF, 1 h 60 °C microwave irradiation; eluent of flash chromatography hexane/acetone 3:2; yellow oil (0.15 g, 0.29 mmol, 95%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 8.21−8.20 (m, 1H), 7.90−7.80 (m, 5H), 7.56−7.52 (m, 2H), 7.40−7.17 (m, 6H), 6.47 (bs, 2H), 4.29 (t, J = 7.3 Hz, 1H), 3.42−3.34 (m, 2H), 2.51−2.43 (m, 2H) ppm; MS (ESI) m/z: 543.06 [M + Na$^+$].

N-(3-(4-Fluorophenyl)-3-(4-(trifluoromethyl)phenyl)propyl)-3-iodobenzamide ***15t***. Procedure E; 0.20 g (0.79 mmol) **17a**, 0.24 g (0.79 mmol) **10q**, 0.45 g (0.87 mmol) PyBOP, 0.06 g (0.40 mmol) HOBt·H_2O, 0.3 mL (1.58 mmol) DIPEA, 35 mL THF, room temperature 16 h; eluent of column chromatography hexane/acetone 2:1; yellow solid (0.31 g, 0.58 mmol, 74%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 8.20 (td, J = 1.7, 0.4 Hz, 1H), 7.92−7.75 (m, 3H), 7.69−7.54 (m, 4H), 7.44−7.39 (m, 2H), 7.26 (t, J = 7.8 Hz, 1H), 7.10−7.04 (m, 2H), 4.29 (t, J = 7.8 Hz, 1H), 3.42−3.34 (m, 2H), 2.50−2.42 (m, 2H) ppm; MS (ESI) m/z: 550.00 [M + Na$^+$].

N-(3-(4-Fluorophenyl)-3-(4-(trifluoromethoxy)phenyl)propyl)-3-iodobenzamide ***15u***. Procedure E; 0.27 g (1.06 mmol) **17a**, 0.33 g (1.06 mmol) **10r**, 0.61 g (1.16 mmol) PyBOP, 0.08 g (0.53 mmol) HOBt·H_2O, 0.4 mL (1.12 mmol) DIPEA, 8 mL THF, 1 h 60 °C microwave irradiation; eluent of column chromatography hexane/ethyl acetate 9:1−3:1; transparent oil (0.55 g, 1.01 mmol, 96%); ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 8.56 (t, J = 5.2 Hz, 1H), 8.15 (t, J = 1.6 Hz, 1H), 7.97−7.76 (m, 2H), 7.52−7.20 (m, 7H), 7.12 (t, J = 8.9 Hz, 2H), 4.14 (t, J = 7.4 Hz, 1H), 3.24−3.12 (m, +2H), 2.33−2.23 (m, 2H) ppm; MS (ESI) m/z: 542.10 [M − H$^+$].

N-(3,3-Bis(4-fluorophenyl)propyl)-3-iodobenzamide ***15v***. Procedure E; 0.20 g (0.79 mmol) **17a**, 0.20 g (0.79 mmol) **10s**, 0.45 g (0.87 mmol) PyBOP, 0.06 g (0.40 mmol) HOBt·H_2O, 0.3 mL (1.58 mmol) DIPEA, 35 mL THF, room temperature 18 h; eluent of column chromatography hexane/acetone 2:1; transparent oil (0.32 g, 0.67 mmol, 85%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 8.19 (t, J = 1.6 Hz, 1H), 7.89−7.85 (m, 3H), 7.42−7.23 (m, 5H), 7.10−7.00 (m, 4H), 4.17 (t, J = 7.8 Hz, 1H), 3.40−3.31 (m, 2H), 2.45−2.34 (m, 2H) ppm; MS (ESI) m/z: 500.07 [M + Na$^+$].

N-(2-(Diphenylamino)ethyl)-3-iodobenzamide ***15x***. Procedure E; 0.60 g (0.24 mmol) **17a**, 0.50 g (0.24 mmol) **10t**, 0.14 g (0.26 mmol) PyBOP, 0.02 g (0.12 mmol) HOBt·H_2O, 0.1 mL (0.47 mmol)

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DIPEA, 30 mL THF, room temperature 24 h; eluent of column chromatography hexane/ethyl acetate 3:1−2:1; transparent oil (0.10 g, 0.23 mmol, 96%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 8.17 (t, J = 1.6 Hz, 1H), 8.01 (bs, 1H), 7.90−7.83 (m, 2H), 7.31−7.23 (m, 5H), 7.11−7.06 (m, 4H), 6.97−6.91 (m, 2H), 4.02−3.96 (m, 2H), 3.72−3.62 (m, 2H) ppm; MS (ESI) m/z: 465.10 [M + Na$^+$].

(R)-N-(3-(4-Fluorophenyl)-3-(4-(trifluoromethyl)phenyl)propyl)-3-iodobenzamide ***15y***. Procedure E; 43 mg (0.17 mmol) **17a**, 50 mg (0.17 mmol) **10u** ((*R*)-3-(4-fluorophenyl)-3-(4-(trifluoromethyl)-phenyl)propan-1-amine), 131 mg (0.25 mmol) PyBOP, 40 mg (0.25 mmol) HOBt·H_2O, 0.1 mL (0.50 mmol) DIPEA, 10 mL THF, room temperature 18 h; eluent of column chromatography hexane/ethyl acetate 9:1−3:1; transparent oil (55 mg, 0.10 mmol, 63%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 8.19 (t, J = 1.6 Hz, 1H), 7.90−7.84 (m, 3H), 7.67−7.56 (m, 4H), 7.46−7.39 (m, 2H), 7.26 (t, J = 7.8 Hz, 1H), 7.11−7.03 (m,2H), 4.30 (t, J = 7.8 Hz, 1H), 3.43−3.33 (m, 2H), 2.51−2.41 (m, 2H) ppm; MS (ESI) m/z: 549.96 [M + Na$^+$].

5-Bromo-N-(2-(trifluoromethyl)benzyl)nicotinamide ***19***. Under an argon atmosphere, 0.24 g of (1.17 mmol, 1 equiv) **18** and 0.67 g of (1.76 mmol, 1.5 equiv) HBTU were dissolved in 15 mL of dry DMF and cooled to 0 °C. To the solution, 0.23 g of (1.29 mmol, 1.1 equiv) **10a**, 0.7 mL of (7.02 mmol, 6 equiv) 4-methylmorpholine, and 0.34 g of (1.76 mmol, 1.5 equiv) EDC·HCl were added. The solution was stirred for 3 days at room temperature. The solvent was evaporated, and the residue was suspended in water. The aqueous phase was extracted with ethyl acetate (4×), and the combined organic phase was washed with brine. After drying over $MgSO_4$ and filtration, the solvent was removed, and the crude product was purified via flash chromatography (hexane/ethyl acetate 2:1). A white solid was obtained (0.27 g, 0.75 mmol, 64%); ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 9.39 (t, J = 5.6 Hz, 1H), 9.04 (d, J = 1.8 Hz, 1H), 8.89 (d, J = 2.2 Hz, 1H), 8.50 (t, J = 2.1 Hz, 1H), 7.77−7.46 (m, 4H), 4.67 (d, J = 5.5 Hz, 2H) ppm; MS (ESI) m/z: 358.90 [M + H$^+$].

5-Iodo-N-(2-(trifluoromethyl)benzyl)nicotinamide ***15z***. Under an argon atmosphere, 260 mg of (0.72 mmol, 1 equiv) **19**, 217 mg of (1.45 mmol, 2 equiv) dry NaI, and 7 mg of (0.04 mmol, 0.05 equiv) CuI were suspended in 3 mL of dry 1,4-dioxane. 8 μL of (0.08 mmol, 0.1 equiv) *trans*-1,2-cyclohexanediamine was added, and it was heated to 155 °C via microwave irradiation for 8 h. The suspension was allowed to cool to room temperature and diluted with ethyl acetate and aqueous ammonia (10%). The aqueous phase was extracted with ethyl acetate (3×), and the combined organic phase was dried over $MgSO_4$ and filtered. The solvent was evaporated, and the crude product was used without further purification.

5-Bromo-N-(2-(trifluoromethyl)benzyl)furan-2-carboxamide ***21***. **20** (0.17 g, 0.87 mmol, 1 equiv) was dissolved in 10 mL of dry DMF, and 0.3 mL of (2.28 mmol, 2.6 equiv) **10a**, 0.7 mL of (3.90 mmol, 4.4 equiv) DIPEA, and 0.54 g of (1.04 mmol, 1.2 equiv) PyBOP were added. The solution was stirred for 2 days at room temperature. The mixture was concentrated and diluted with DCM. The organic phase was washed with saturated aqueous $NaHCO_3$ solution (3×), dried over $MgSO_4$, and filtered. The solvent was evaporated, and the residue was purified with flash chromatography (hexane/ethyl acetate 3:1) yielding a brownish solid (0.17 g, 0.48 mmol, 55%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.05 (t, J = 4.8 Hz, 1H), 7.74−7.63 (m, 2H), 7.51−7.45 (m, 2H), 7.22 (d, J = 3.6 Hz, 1H), 6.79 (d, J = 3.6 Hz, 1H), 4.60 (d, J = 4.8 Hz, 2H) ppm; MS (ESI) m/z: 349.70 [M + H$^+$].

5-Iodo-N-(2-(trifluoromethyl)benzyl)furan-2-carboxamide ***15aa***. Under an argon atmosphere, 150 mg of (0.43 mmol, 1 equiv) **21**, 129 mg of (0.86 mmol, 2 equiv) dry NaI, and 4 mg of (0.02 mmol, 0.05 equiv) CuI were suspended in 3 mL of dry 1,4-dioxane. 6 μL of (0.04 mmol, 0.1 equiv) *trans*-1,2-cyclohexanediamine was added, and the mixture was heated to 110 °C via microwave irradiation for 21 h. The suspension was allowed to cool to room temperature and diluted with ethyl acetate and aqueous ammonia (10%). The aqueous phase was extracted with ethyl acetate (3×), the combined organic phase was dried over $MgSO_4$ and filtered. The solvent was evaporated, and the crude product was used without further purification.

General Procedure for the Synthesis of Target Structures 7a-7af (Procedure F). The iodo derivative (1 equiv), 1.1 equiv of alkyne derivative, 0.02 equiv of bis(acetonitrile)dichloropalladium (II), 0.04 equiv of CuI, and 0.04 equiv of PPh_3 were suspended in abs ethyl acetate. Solutions of 1−12 equiv of DIPA were added, and the mixture stirred for 36−48 h at room temperature. The suspension was washed with water (3×), and the combined aqueous phase was extracted with ethyl acetate (1×). The combined organic phase was washed with brine (1×) and dried over $MgSO_4$. The $MgSO_4$ was filtered off, and the solvent was evaporated under reduced pressure. The residue was purified with column chromatography, and most derivatives were purified further with semipreparative HPLC to gain a purity over 95%.

4-(3-(1-Hydroxyureido)but-1-yn-1-yl)-N-(2-(trifluoromethyl)-benzyl)benzamide ***7a***. Procedure F; 320 mg (0.79 mmol) **15a**, 110 mg (0.78 mmol) **8a**, 4 mg (0.02 mmol) $Pd(ACN)_2Cl_2$, 6 mg (0.03 mmol) CuI, 8 mg (0.03 mmol) PPh_3, 0.1 mL (0.95 mmol, 1.2 equiv) DIPA, 18 mL ethyl acetate, 36 h, eluent of flash chromatography hexane/ethyl acetate 1:2, orange solid (280 mg, 0.69 mmol, 88%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.38 (s, 1H), 9.17 (t, J = 5.8 Hz, 1H), 7.94−7.89 (m, 2H), 7.76−7.61 (m, 2H), 7.56−7.46 (m, 4H), 6.56 (s, 2H), 5.17 (q, J = 7.0 Hz, 1H), 4.66 (d, J = 5.4 Hz, 2H), 1.38 (d, J = 7.0 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 165.8, 161.4, 137.4, 137.3, 133.4, 132.7, 131.4, 129.3, 128.3, 127.6, 127.3, 126.3, 125.9, 125.7 (q), 125.5, 92.2, 81.2, 45.9, 18.4 ppm; purity (HPLC-MS): 99%, t_R: 3.46 min (method: gradient of 50% ACN to 90% within 10 min); HRMS (MALDI): m/z calculated for $C_{20}H_{18}F_3N_3O_3$ + H$^+$ [M + H$^+$]: 406.13730; found: 406.13664.

3-(3-(1-Hydroxyureido)but-1-yn-1-yl)-N-(2-(trifluoromethyl)-benzyl)benzamide ***7b***. Procedure F; 120 mg (0.30 mmol) **15b**, 40 mg (0.33 mmol) **8a**, 2 mg (0.01 mmol) $Pd(ACN)_2Cl_2$, 2 mg (0.01 mmol) CuI, 3 mg (0.01 mmol) PPh_3, 0.1 mL (0.36 mmol, 1.2 equiv) DIPA, 8 mL ethyl acetate, 20 h, further purification with preparative HPLC , white solid (96 mg, 0.24 mmol, 79%); ^{1}H-NMR (400 MHz, DMSO-d_6) δ = 9.36 (s, 1H), 9.21 (t, J = 5.6 Hz, 1H), 7.97 (s, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.80−7.41 (m, 6H), 6.56−6.53 (m, 2H), 5.16 (q, J = 7.0 Hz, 1H), 4.67−4.63 (m, 2H), 1.38 (d, J = 7.0 Hz, 3H) ppm; ^{13}C-NMR (101 MHz, DMSO-d_6) δ = 165.7, 161.4, 137.4, 134.3, 134.2, 132.7, 130.1, 128.8, 128.3, 127.4, 127.3, 125.8 (q), 122.7, 90.7, 81.08, 45.8, 18.5 ppm; purity (UPLC-MS): 99%, t_R: 3.57 min (method: gradient of 25% ACN to 90% within 5 min); HRMS (MALDI): m/z calculated for $C_{20}H_{18}F_3N_3O_3$ + H$^+$ [M + H$^+$]: 406.13730; found: 406.13709.

5-(3-(1-Hydroxyureido)but-1-yn-1-yl)-N-(2-(trifluoromethyl)-benzyl)nicotinamide ***7c***. Procedure F; 140 mg (0.35 mmol) **15z**, 50 mg (0.40 mmol) **8a**, 2 mg (0.01 mmol) $Pd(ACN)_2Cl_2$, 3 mg (0.01 mmol) CuI, 4 mg (0.01 mmol) PPh_3, 0.1 mL (0.43 mmol, 1.2 equiv) DIPA, 10 mL ethyl acetate, 49 h, further purification with flash chromatography (hexane/ethyl acetate 1:1);white solid (110 mg, 0.27 mmol, 76%); ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 9.44−9.37 (m, 2H), 9.01 (s, 1H), 8.74 (s, 1H), 8.30 (t, J = 2.0 Hz, 1H), 7.76−7.45 (m, 4H), 6.60 (s, 2H), 5.20 (q, J = 7.0 Hz, 1H), 4.67 (d, J = 5.4 Hz, 2H), 1.40 (d, J = 7.0 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 164.3, 161.4, 153.8, 147.7, 137.3, 137.0 (d), 132.8, 129.1, 128.7, 127.5, 126.4 (d), 126.0, 125.8 (q), 122.7, 119.2, 94.1, 78.1, 46.0, 18.4 ppm; purity (UPLC-MS): 97%, t_R: 2.98 min (method: gradient of 25% ACN to 90% within 5 min); HRMS (MALDI): m/z calculated for $C_{19}H_{17}F_3N_4O_3$ + H$^+$ [M + H$^+$]: 407.13255; found: 407.13251.

5-(3-(1-Hydroxyureido)but-1-yn-1-yl)-N-(2-(trifluoromethyl)-benzyl)furan-2-carboxamide ***7d***. Procedure F; 140 mg (0.35 mmol) **15aa**, 50 mg (0.40 mmol) **8a**, 2 mg (0.01 mmol) $Pd(ACN)_2Cl_2$, 3 mg (0.01 mmol) CuI, 4 mg (0.01 mmol) PPh_3, 0.1 mL (0.43 mmol, 1.2 equiv) DIPA, 10 mL ethyl acetate, 48 h, further purification with preparative HPLC (5 min 30% ACN, gradient from 30% ACN to 80% within 15 min), white solid (4 mg, 0.01 mmol, 3%); ^{1}H-NMR (500 MHz, DMSO-d_6) δ = 9.45 (s, 1H), 9.17 (t, J = 6.0 Hz, 1H), 7.72 (d, J = 7.7 Hz, 1H), 7.67−7.64 (m, 1H), 7.50−7.45 (m, 2H), 7.18 (d, J = 3.5 Hz, 1H), 6.90 (d, J = 3.6 Hz, 1H), 6.61 (s, 2H), 5.18 (q, J = 7.1 Hz, 1H), 4.59 (d, J = 5.8 Hz, 2H), 1.38 (d, J = 7.1 Hz, 3H) ppm; ^{13}C-NMR (101 MHz, DMSO-d_6) δ = 161.9, 157.7, 148.2, 137.7, 137.7,

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133.2, 129.2, 128.6, 127.8, 126.6, 126.3, 126.2 (q), 126.0, 123.9, 117.5, 115.4, 96.2, 72.3, 46.4, 18.8 ppm; purity (UPLC-MS): 97%, t_R: 3.39 min (method: gradient of 20% ACN to 90% within 5 min); HRMS (MALDI): *m/z* calculated for $C_{18}H_{16}F_3N_3O_4 + K^+$ [M + K^+]: 434.07245; found: 434.07242.

3-(3-(1-Hydroxyureido)but-1-yn-1-yl)-4-methyl-N-(2-(trifluoromethyl)benzyl)-benzamide ***7e***. Procedure F; 90 mg (0.22 mmol) **15l**, 31 mg (0.24 mmol) **8a**, 1 mg (0.004 mmol, 0.03 equiv) $Pd(ACN)_2Cl_2$, 2 mg (0.01 mmol, 0.04 equiv) CuI, 5 mg (0.01 mmol, 0.09 equiv) PPh_3, 0.1 mL (0.26 mmol, 1.2 equiv) DIPA, 12 mL ethyl acetate, 39 h, further purification with column chromatography (ethyl acetate 100%); yellow solid (42 mg, 0.10 mmol, 47%); ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 9.36 (s, 1H), 9.15 (t, J = 5.7 Hz, 1H), 7.94 (d, J = 1.8 Hz, 1H), 7.85−7.33 (m, 6H), 6.59 (s, 2H), 5.18 (q, J = 7.0 Hz, 1H), 4.65 (d, J = 5.4 Hz, 2H), 2.41 (s, 3H), 1.41 (d, J = 7.0 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 165.7, 161.7, 143.3, 137.6 (d), 132.7, 131.6, 130.2, 129.6, 128.3, 127.3, 126.3, 125.9, 125.7 (q), 122.5, 94.6, 80.0, 46.1, 20.2, 18.8 ppm; purity (UPLC-MS): 98% t_R: 3.99 min (method: gradient of 20% ACN to 90% within 5 min); HRMS (MALDI): *m/z* calculated $C_{21}H_{20}F_3N_3O_3 + H^+$ [M + H^+]: 420.15295; found: 420.15267.

3-(3-(1-Hydroxyureido)prop-1-yn-1-yl)-N-(2-(trifluoromethyl)-benzyl)benzamide ***7f***. Procedure F; 100 mg (0.25 mmol) **15b**, 30 mg (0.27 mmol) **8b**, 1 mg (0.005 mmol) $Pd(ACN)_2Cl_2$, 2 mg (0.01 mmol) CuI, 3 mg (0.01 mmol) PPh_3, 0.1 mL (0.30 mmol, 1.2 equiv) DIPA, 15 mL ethyl acetate, 39 h, further purification with column chromatography (hexane/ethyl acetate 4:1−0:1);brown solid (50 mg, 0.13 mmol, 52%); ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 9.62 (s, 1H), 9.23 (t, J = 5.7 Hz, 1H), 8.00 (t, J = 1.4 Hz, 1H), 7.92 (td, J = 1.3, 7.8 Hz, 1H), 7.77−7.43 (m, 6H), 6.56 (s, 2H), 4.66 (d, J = 5.4 Hz, 2H), 4.35 (s, 2H) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 165.7, 161.6, 137.4, 134.3 (d), 132.7, 132.1, 131.5 (d), 130.2, 128.9, 128.8, 128.7, 128.3, 127.5, 127.3, 126.3, 125.8 (q), 122.6, 87.1, 81.8 ppm; purity (HPLC-MS): 97% t_R: 7.62 min (method: gradient of 30% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{19}H_{16}F_3N_3O_3 + H^+$ [M + H^+]: 392.12165; found: 392.12133.

(S)-3-(3-(1-Hydroxyureido)but-1-yn-1-yl)-N-(2-(trifluoromethyl)-benzyl)benzamide ***7g***. Procedure F; 80 mg (0.20 mmol) **15b**, 30 mg (0.22 mmol) **8c**, 1 mg (0.004 mmol) $Pd(ACN)_2Cl_2$, 2 mg (0.01 mmol) CuI, 3 mg (0.01 mmol) PPh_3, 0.1 mL (0.24 mmol, 1.2 equiv) DIPA, 11 mL ethyl acetate, 43 h, further purification with column chromatography (hexane/acetone 2:3), further purification with preparative HPLC (5% ACN to 90% within 10 min, 90% ACN for 6 min); white solid (27 mg, 0.07 mmol, 34%); ^{1}H-NMR (500 MHz, acetone-d_6) δ = 8.69 (s, 1H), 8.42 (t, J = 5.3 Hz, 1H), 8.05−7.91 (m, 2H), 7.73 (d, J = 7.9 Hz, 1H), 7.70−7.54 (m, 3H), 7.53−7.43 (m, 2H), 6.13 (s, 2H), 5.27 (q, J = 7.0 Hz, 1H), 4.83 (d, J = 5.7 Hz, 2H), 1.45 (d, J = 7.0 Hz, 3H) ppm; ^{13}C-NMR (125 MHz, acetone-d_6) δ = 166.8, 162.0, 135.7, 133.4, 131.2, 129.8, 129.5, 128.2, 128.0, 126.6 (q), 124.4, 90.8, 82.2, 47.3, 40.6 (q), 18.6 ppm; purity (HPLC-MS): 97% t_R: 9.66 min (method: gradient of 10% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{20}H_{18}F_3N_3O_3 + H^+$ [M + H^+]: 406.13730; found: 406.13715.

(R)-3-(3-(1-Hydroxyureido)but-1-yn-1-yl)-N-(2-(trifluoromethyl)-benzyl)benzamide ***7h***. Procedure F; 80 mg (0.20 mmol) **15b**, 30 mg (0.22 mmol) **8d**, 1 mg (0.004 mmol) $Pd(ACN)_2Cl_2$, 2 mg (0.01 mmol) CuI, 3 mg (0.01 mmol) PPh_3, 0.1 mL (0.24 mmol, 1.2 equiv) DIPA, 15 mL ethyl acetate, 43 h, further purification with column chromatography (hexane/acetone 2:3), further purification with preparative HPLC (linear gradient from 5% ACN to 90% within 10 min, 90% ACN for 6 min); white solid (29 mg, 0.07 mmol, 36%); ^{1}H-NMR (500 MHz, acetone-d_6) δ = 8.69 (s, 1H), 8.42 (t, J = 5.4 Hz, 1H), 8.02−7.91 (m, 2H), 7.76−7.52 (m, 4H), 7.52−7.43 (m, 2H), 6.14 (s, 2H), 5.27 (q, J = 7.0 Hz, 1H), 4.83 (d, J = 5.7 Hz, 2H), 1.45 (d, J = 7.0 Hz, 3H) ppm; ^{13}C-NMR (125 MHz, acetone-d_6) δ = 166.9, 162.0, 138.6, 138.6, 135.7, 135.1, 133.4, 131.2, 129.8, 129.5, 128.2, 128.0, 126.6 (q), 124.4, 90.8, 82.2, 47.3, 40.6 (q), 18.6 ppm; purity (HPLC-MS): 97% t_R: 9.65 min (method: gradient of 10% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{20}H_{18}F_3N_3O_3 + H^+$ [M + H^+]: 406.13730; found: 406.13723.

3-(3-(1-Hydroxyureido)pent-1-yn-1-yl)-N-(2-(trifluoromethyl)-benzyl)benzamide ***7i***. Procedure F; 80 mg (0.20 mmol) **15b**, 30 mg (0.22 mmol) **8e**, 1 mg (0.004 mmol) $Pd(ACN)_2Cl_2$, 2 mg (0.01 mmol) CuI, 3 mg (0.01 mmol) PPh_3, 0.1 mL (0.24 mmol, 1.2 equiv) DIPA, 15 mL ethyl acetate, 43 h, further purification with column chromatography (hexane/acetone 2:3), further purification with preparative HPLC (linear gradient from 5% ACN to 90% within 10 min, 90% ACN for 6 min);white solid (58 mg, 0.14 mmol, 70%); ^{1}H-NMR (500 MHz, acetone-d_6) δ = 8.67 (s, 1H), 8.43 (t, J = 6.0 Hz, 1H), 8.00 (t, J = 1.5 Hz, 1H), 7.94 (td, J = 1.5, 7.8 Hz, 1H), 7.74−7.71 (m, 1H), 7.69−7.55 (m, 3H), 7.53−7.43 (m, 2H), 6.13 (s, 2H), 5.05 (t, J = 7.8 Hz, 1H), 4.83 (d, J = 5.7 Hz, 2H), 1.91−1.83 (m, 2H), 1.02 (t, J = 7.4 Hz, 3H) ppm; ^{13}C-NMR (125 MHz, acetone-d_6) δ = 166.9, 162.3, 138.6, 138.6, 135.7, 135.1, 133.3, 131.2, 129.8, 129.5, 128.2, 128.0, 126.7 (q), 126.6, 124.4, 89.9, 83.0, 53.3, 40.6 (q), 26.6, 11.1 ppm; purity (HPLC-MS): 97% t_R: 10.03 min (method: gradient of 10% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{21}H_{20}F_3N_3O_3 + H^+$ [M + H^+]: 420.15295; found: 420.15279.

3-(3-(1-Hydroxyureido)hex-1-yn-1-yl)-N-(2-(trifluoromethyl)-benzyl)benzamide ***7j***. Procedure F; 80 mg (0.20 mmol) **15b**, 30 mg (0.22 mmol) **8f**, 1 mg (0.004 mmol) $Pd(ACN)_2Cl_2$, 2 mg (0.01 mmol) CuI, 2 mg (0.01 mmol) PPh_3, 0.1 mL (0.24 mmol, 1.2 equiv) DIPA, 15 mL ethyl acetate, 43 h, further purification with column chromatography (hexane/acetone 2:3), further purification with preparative HPLC (linear gradient from 5% ACN to 90% within 10 min, 90% ACN for 6 min);white solid (57 mg, 0.13 mmol, 67%); ^{1}H-NMR (500 MHz, acetone-d_6) δ = 8.65 (s, 1H), 8.43 (t, J = 5.5 Hz, 1H), 8.12−7.76 (m, 2H), 7.73 (d, J = 7.9 Hz, 1H), 7.69−7.55 (m, 3H), 7.52−7.34 (m, 2H), 6.12 (s, 2H), 5.16 (t, J = 7.7 Hz, 1H), 4.83 (d, J = 5.7 Hz, 2H), 1.83 (q, J = 7.7 Hz, 2H), 1.53−1.45 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H) ppm; ^{13}C-NMR (125 MHz, acetone-d_6) δ = 166.9, 162.2, 138.6, 138.6, 135.7, 135.1, 133.3, 131.2, 129.8, 129.5, 128.2, 128.0, 126.8, 126.6 (q), 124.6, 124.4, 90.0, 82.9, 51.4, 40.6 (q), 35.3, 20.0, 13.9 ppm; purity (HPLC-MS): 97% t_R: 10.51 min (method: gradient of 10% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{22}H_{22}F_3N_3O_3 + Na^+$ [M + Na^+]: 456.15055; found: 456.15027.

3-(3-(1-Hydroxyureido)-4-methylpent-1-yn-1-yl)-N-(2-(trifluoromethyl)benzyl)-benzamide ***7k***. Procedure F; 100 mg (0.25 mmol) **15b**, 42 mg (0.27 mmol) **8g**, 1 mg (0.005 mmol) $Pd(ACN)_2Cl_2$, 6 mg (0.03 mmol, 0.13 equiv) CuI, 11 mg (0.04 mmol, 0.17 equiv) PPh_3, 0.1 mL (0.30 mmol, 1.2 equiv) DIPA, 15 mL ethyl acetate, 39 h, further purification with column chromatography (ethyl acetate 100%), further purification with preparative HPLC (30% ACN for 3 min, linear gradient from 30% ACN to 80% within 8 min, 90% ACN for 6 min); white solid (20 mg, 0.05 mmol, 19%); ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 9.36−9.15 (m, 2H), 8.06−7.15 (m, 7H), 6.53 (s, 2H), 4.79−4.55 (m, 3H), 3.68−3.11 (m, 1H), 2.12−1.97 (m, 1H), 1.10 (d, J = 6.8 Hz, 3H), 0.95 (d, J = 6.5 Hz, 3H) ppm; ^{13}C-NMR (125 MHz, DMSO-d_6) δ = 165.7, 161.8, 137.5 (d), 134.3, 134.2, 132.8, 130.1, 128.9, 128.3, 127.4 (d), 126.3, 125.8 (q), 122.8, 89.3, 82.5, 56.7, 30.5, 20.0, 19.4 ppm; purity (UPLC-MS): 99% t_R: 3.93 min (method: gradient of 20% ACN to 90% within 5 min); HRMS (MALDI): *m/z* calculated $C_{22}H_{22}F_3N_3O_3 + H^+$ [M + H^+]: 434.16860; found: 434.16810.

3-(3-(1-Hydroxyureido)-5-methylhex-1-yn-1-yl)-N-(2-(trifluoromethyl)benzyl)-benzamide ***7l***. Procedure F; 100 mg (0.25 mmol) **15b**, 46 mg (0.27 mmol) **8h**, 1 mg (0.005 mmol, 0.03 equiv) $Pd(ACN)_2Cl_2$, 2 mg (0.01 mmol) CuI, 11 mg (0.04 mmol, 0.17 equiv) PPh_3, 0.1 mL (0.30 mmol, 1.2 equiv) DIPA, 15 mL ethyl acetate, 39 h, further purification with column chromatography (DCM/MeOH 9:1); yellow solid (62 mg, 0.14 mmol, 56%); ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 9.33 (s, 1H), 9.24 (t, J = 5.8 Hz, 1H), 7.97−7.87 (m, 2H), 7.76−7.44 (m, 6H), 6.56 (s, 2H), 5.09 (t, J = 7.8 Hz, 1H), 4.65 (d, J = 5.4 Hz, 2H), 1.81−1.59 (m, 3H), 0.92 (dd, J = 3.5, 6.5 Hz, 6H) ppm; ^{13}C-NMR (125 MHz, DMSO-d_6) δ = 165.7, 161.6, 137.4, 134.3, 134.2, 132.7, 130.1, 128.9, 128.3127.4 (d),

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126.3, 125.8 (q), 122.7, 89.8, 81.7, 48.5, 40.9, 24.3, 22.2 (d) ppm; purity (HPLC-MS): 96% t_R: 10.96 min (method: gradient of 10% ACN to 90% within 10 min, hold 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{23}H_{24}F_3N_3O_3$ + H^+ [M + H^+]: 448.18425; found: 448.18368.

N-(2-(1H-Indol-3-yl)ethyl)-3-(3-(1-hydroxyureido)but-1-yn-1-yl)-benzamide ***7m***. Procedure F; 150 mg (0.38 mmol) **15c**, 52 mg (0.41 mmol) **8a**, 3 mg (0.01 mmol, 0.03 equiv) $Pd(ACN)_2Cl_2$, 5 mg (0.03 mmol, 0.07 equiv) CuI, 5 mg (0.02 mmol) PPh_3, 0.1 mL (0.44 mmol, 1.2 equiv) DIPA, 16 mL ethyl acetate, 24 h, purification with flash chromatography (DCM/MeOH 100:0−9:1), further purification with preparative HPLC (linear gradient from 20% ACN to 90% within 10 min, 90% ACN for 10 min); white solid (47 mg, 0.12 mmol, 34%); ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 10.80 (s, 1H), 9.37 (s, 1H), 8.72 (t, *J* = 5.6 Hz, 1H), 7.89−7.81 (m, 2H), 7.60−7.42 (m, 3H), 7.36−7.31 (m, 1H), 7.17 (d, *J* = 2.2 Hz, 1H), 7.10−6.94 (m, 2H), 6.56 (s, 2H), 5.15 (q, *J* = 7.0 Hz, 1H), 3.58−3.48 (m, 2H), 2.94 (t, *J* = 7.4 Hz, 2H), 1.38 (d, *J* = 7.0 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 136.2, 135.0, 130.0, 128.7, 127.3, 122.6, 120.9, 118.22, 111.9, 111.4, 45.8, 25.1, 18.6 ppm; purity (UPLC-MS): 99% t_R: 3.03 min (method: gradient of 20% ACN to 90% within 5 min); HRMS (MALDI): *m/z* calculated $C_{22}H_{22}N_4O_3$ + H^+ [M + H^+]: 391.17647; found: 391.17631.

N-(4-Fluorophenethyl)-3-(3-(1-hydroxyureido)but-1-yn-1-yl)-benzamide ***7n***. Procedure F; 80 mg (0.21 mmol) **15d**, 29 mg (0.23 mmol) **8a**, 2 mg (0.007 mmol, 0.03 equiv) $Pd(ACN)_2Cl_2$, 2 mg (0.01 mmol, 0.06 equiv) CuI, 3 mg (0.01 mmol, 0.06 equiv) PPh_3, 0.1 mL (0.25 mmol, 1.2 equiv) DIPA, 9 mL ethyl acetate, 20 h, purification with preparative HPLC (30% ACN for 5 min, linear gradient from 30% ACN to 80% within 10 min); white solid (30 mg, 0.08 mmol, 39%); ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 9.37 (s, 1H), 8.65 (t, *J* = 5.5 Hz, 1H), 7.84−7.75 (m, 2H), 7.54−7.41 (m, 2H), 7.30−7.23 (m, 2H), 7.14−7.07 (m, 2H), 6.55 (s, 2H), 5.15 (q, *J* = 7.0 Hz, 1H), 3.51−3.41 (m, 2H), 2.83 (t, *J* = 7.2 Hz, 2H), 1.38 (d, *J* = 7.0 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 165.3, 161.5, 135.7 (d), 134.8, 133.8, 130.5, 130.4, 129.9, 128.7, 127.2, 122.5, 115.1, 114.8, 90.6, 81.1, 45.8, 40.9, 34.1, 18.5 ppm; purity (UPLC-MS): 99% t_R: 2.89 min (method: gradient of 20% ACN to 90% within 5 min); HRMS (MALDI): *m/z* calculated $C_{20}H_{20}FN_3O_3$ + H^+ [M + H^+]: 370.15615; found: 370.15644.

N-(3-Fluorobenzyl)-3-(3-(1-hydroxyureido)but-1-yn-1-yl)-benzamide ***7o***. Procedure F; 70 mg (0.21 mmol) **15e**, 29 mg (0.23 mmol) **8a**, 2 mg (0.007 mmol, 0.03 equiv) $Pd(ACN)_2Cl_2$, 2 mg (0.01 mmol, 0.05 equiv) CuI, 3 mg (0.01 mmol, 0.06 equiv) PPh_3, 0.1 mL (0.25 mmol, 1.2 equiv) DIPA, 10 mL ethyl acetate, 41 h, purification with preparative HPLC (30% ACN for 5 min, linear gradient from 30% ACN to 80% within 10 min); white solid (16 mg, 0.05 mmol, 22%); ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 9.38 (s, 1H), 9.21 (t, *J* = 5.9 Hz, 1H), 7.99−7.79 (m, 2H), 7.61−7.31 (m, 3H), 7.23−6.98 (m, 3H), 6.59 (s, 2H), 5.15 (q, *J* = 7.0 Hz, 1H), 4.47 (d, *J* = 5.9 Hz, 2H), 1.37 (d, *J* = 7.0 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 165.5, 142.6 (d), 134.5, 134.0, 130.3 (d), 130.1, 128.9, 127.4, 123.3, 122.6, 114.1, 113.8 (d), 113.4, 90.6, 81.1, 45.8, 42.3, 18.5 ppm; purity (UPLC-MS): 98% t_R: 2.93 min (method: gradient of 20% ACN to 90% within 5 min); HRMS (MALDI): *m/z* calculated $C_{19}H_{18}FN_3O_3$ + K^+ [M + K^+]: 394.09638; found: 394.09612.

3-(3-(1-Hydroxyureido)but-1-yn-1-yl)-N-(2-(trifluoromethoxy)-benzyl)benzamide ***7p***. Procedure F; 98 mg (0.23 mmol) **15k**, 33 mg (0.26 mmol, 1.2 equiv) **8a**, 2 mg (0.01 mmol, 0.03 equiv) $Pd(ACN)_2Cl_2$, 2 mg (0.02 mmol, 0.07 equiv) CuI, 6 mg (0.02 mmol, 0.1 equiv) PPh_3, 0.1 mL (0.30 mmol, 1.2 equiv) DIPA, 10 mL ethyl acetate, 5 mL THF, 41 h, purification with column chromatography (hexane/ethyl acetate 4:1), further purification with preparative HPLC (linear gradient from 10% ACN to 90% within 10 min); off-white solid (60 mg, 0.14 mmol, 61%); ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 9.36 (s, 1H), 9.15 (t, *J* = 5.7 Hz, 1H), 7.94−7.85 (m, 2H), 7.58−7.34 (m, 6H), 6.56 (s, 2H), 5.15 (q, *J* = 7.0 Hz, 1H), 4.53 (d, *J* = 5.9 Hz, 2H), 1.37 (d, *J* = 7.1 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 165.5, 161.4, 146.1 (d), 134.4, 134.1, 131.7, 130.1, 129.3, 128.8, 128.7, 127.5, 122.6, 120.4 (q), 90.6, 81.1, 45.8, 18.5 ppm; purity (HPLC-MS): 96% t_R: 9.86 min (method: gradient of 10% ACN to 90% within 10 min); HRMS (MALDI): *m/z* calculated $C_{20}H_{18}F_3N_3O_4$ + H^+ [M + H^+]: 422.13222; found: 422.13171.

N-(2,4-Dichlorobenzyl)-3-(3-(1-hydroxyureido)but-1-yn-1-yl)-benzamide ***7q***. Procedure F; 75 mg (0.19 mmol) **15f**, 27 mg (0.23 mmol, 1.2 equiv) **8a**, 2 mg (0.01 mmol, 0.04 equiv) $Pd(ACN)_2Cl_2$, 2 mg (0.01 mmol, 0.06 equiv) CuI, 3 mg (0.01 mmol, 0.07 equiv) PPh_3, 0.1 mL (0.22 mmol, 1.2 equiv) DIPA, 9 mL ethyl acetate, 20 h, purification with preparative HPLC (linear gradient from 50% ACN to 80% within 10 min); white solid (41 mg, 0.10 mmol, 55%); ^{1}H-NMR (500 MHz, DMSO-d_6) δ = 9.37 (s, 1H), 9.17 (t, *J* = 5.7 Hz, 1H), 7.94 (s, 1H), 7.87 (dd, *J* = 1.3, 7.8 Hz, 1H), 7.63−7.60 (m, 1H), 7.59−7.54 (m, 1H), 7.49 (t, *J* = 7.7 Hz, 1H), 7.43−7.36 (m, 2H), 6.55 (s, 2H), 5.15 (q, *J* = 7.0 Hz, 1H), 4.50 (d, *J* = 5.7 Hz, 2H), 1.38 (d, *J* = 7.0 Hz, 3H) ppm; ^{13}C-NMR (125 MHz, DMSO-d_6) δ = 165.6, 161.5, 135.4, 134.2 (d), 132.9, 132.3 (d), 132.1 (d), 131.5, 131.4, 130.2 (d), 128.9 (d), 128.7, 128.6, 127.4 (d), 122.7, 90.7, 81.1, 45.8, 18.6 ppm; purity (UPLC-MS): 99% t_R: 3.37 min (method: gradient of 20% ACN to 90% within 5 min); HRMS (MALDI): *m/z* calculated $C_{19}H_{17}Cl_2N_3O_3$ + H^+ [M + H^+]: 406.07197; found: 406.07188.

3-(3-(1-Hydroxyureido)but-1-yn-1-yl)-N-(4-methoxy-2-(trifluoromethyl)benzyl)-benzamide ***7r***. Procedure F; 74 mg (0.17 mmol) **15g**, 29 mg (0.23 mmol, 1.3 equiv) **8a**, 2 mg (0.007 mmol, 0.04 equiv) $Pd(ACN)_2Cl_2$, 2 mg (0.01 mmol, 0.07 equiv) CuI, 3 mg (0.01 mmol, 0.07 equiv) PPh_3, 0.1 mL (0.20 mmol, 1.2 equiv) DIPA, 9 mL ethyl acetate, 20 h, purification with preparative HPLC (30% ACN 1 min, linear gradient from 30% ACN to 80% within 6 min, linear gradient to 90% ACN within 4 min); white solid (22 mg, 0.05 mmol, 30%); ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 9.39 (s, 1H), 9.16 (t, *J* = 5.6 Hz, 1H), 7.97−7.88 (m, 2H), 7.60−7.44 (m, 3H), 7.26−7.22 (m, 2H), 6.58 (s, 2H), 5.17 (q, *J* = 7.0 Hz, 1H), 4.59 (d, *J* = 5.3 Hz, 2H), 3.83 (s, 3H), 1.39 (d, *J* = 7.0 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 165.6, 161.4, 158.0, 134.4, 134.1, 130.5, 130.1, 128.8, 127.4, 127.1, 126.0, 122.6, 122.4, 117.7, 111.5 (q), 90.6, 81.1, 55.6, 45.8, 18.6.ppm; purity (UPLC-MS): 99% t_R: 3.53 min (method: gradient of 20% ACN to 90% within 5 min); HRMS (MALDI): *m/z* calculated $C_{21}H_{20}F_3N_3O_4$ + H^+ [M + H^+]: 436.14787; found: 436.14737.

N-(4-Fluoro-2-(trifluoromethyl)benzyl)-3-(3-(1-hydroxyureido)-but-1-yn-1-yl)benzamide ***7s***. Procedure F; 100 mg (0.24 mmol) **15h**, 33 mg (0.26 mmol) **8a**, 3 mg (0.01 mmol, 0.05 equiv) $Pd(ACN)_2Cl_2$, 4 mg (0.02 mmol, 0.09 equiv) CuI, 7 mg (0.03 mmol, 0.1 equiv) PPh_3, 0.1 mL (0.28 mmol, 1.2 equiv) DIPA, 10 mL ethyl acetate, 5 mL THF, 41 h, purification with column chromatography (hexane/ethyl acetate 1:4), further purification with preparative HPLC (linear gradient from 10% ACN to 90% within 10 min); white solid (65 mg, 0.15 mmol, 65%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 9.36 (s, 1H), 9.21 (t, *J* = 5.6 Hz, 1H), 7.97−7.86 (m, 2H), 7.66−7.45 (m, 5H), 6.54 (s, 2H), 5.16 (q, *J* = 7.0 Hz, 1H), 4.61 (d, *J* = 5.3 Hz, 2H), 1.38 (d, *J* = 7.0 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 165.7, 162.2, 161.5, 158.9, 134.2, 133.6, 131.3 (d), 130.1, 128.9, 127.4, 122.7, 119.6, 119.4, 113.5 (q), 113.2 (q), 90.7, 81.1, 45.8, 18.6 ppm; purity (HPLC-MS): 97% t_R: 9.91 min (method: gradient of 10% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{20}H_{17}F_4N_3O_3$ + H^+ [M + H^+]: 424.12788; found: 424.12847.

N-(3,3-Diphenylpropyl)-3-(3-(1-hydroxyureido)but-1-yn-1-yl)-benzamide ***7t***. Procedure F; 100 mg (0.23 mmol) **15i**, 42 mg (0.26 mmol, 1.5 equiv) **8a**, 3 mg (0.01 mmol, 0.05 equiv) $Pd(ACN)_2Cl_2$, 5 mg (0.02 mmol, 0.11 equiv) CuI, 4 mg (0.02 mmol, 0.07 equiv) PPh_3, 0.1 mL (0.27 mmol, 1.2 equiv) DIPA, 10 mL ethyl acetate, 5 mL THF, 41 h, purification with column chromatography (hexane/ethyl acetate 1:4), further purification with preparative HPLC (30% ACN for 3 min, linear gradient from 30% ACN to 80% within 8 min); off-white solid (48 mg, 0.11 mmol, 48%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 8.89 (s, 1H), 8.11−7.76 (m, 3H), 7.75−6.79 (m, 12H), 6.25−6.10 (m, 2H), 5.28 (q, *J* = 7.0 Hz, 1H), 4.12 (t, *J* = 7.8, 7.8 Hz, 1H), 3.42−3.32 (m, 2H), 2.48−2.37 (m, 2H), 1.45 (d, *J* = 7.0 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, acetone-d_6) δ = 166.6, 162.3, 145.9, 136.2,

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134.7, 131.1, 129.3, 128.7, 127.9, 127.0, 124.2, 90.6, 82.4, 49.8, 47.3, 39.5, 35.9, 18.7 ppm; purity (HPLC-MS): 97% t_R: 10.57 min (method: gradient of 10% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{27}H_{27}N_3O_3$ + H^+ [M + H^+]: 442.21252; found: 442.21247.

N-(3-(4-Fluorophenyl)-3-phenylpropyl)-3-(3-(1-hydroxyureido)-but-1-yn-1-yl)benzamide ***7u***. Procedure F; 114 mg (0.25 mmol) **15j**, 46 mg (0.36 mmol, 1.5 equiv) **8a**, 3 mg (0.01 mmol, 0.05 equiv) $Pd(ACN)_2Cl_2$, 5 mg (0.03 mmol, 0.12 equiv) CuI, 4 mg (0.02 mmol, 0.07 equiv) PPh_3, 0.1 mL (0.30 mmol, 1.2 equiv) DIPA, 10 mL ethyl acetate, 38 h, purification with column chromatography (ethyl acetate 100%), further purification with preparative HPLC (linear gradient from 5% ACN to 90% within 10 min, 90% ACN for 6 min); white solid (35 mg, 0.08 mmol, 34%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 8.68 (s, 1H), 7.87−7.80 (m, 3H), 7.53−7.01 (m, 11H), 6.12 (s, 2H), 5.27 (q, *J* = 7.0 Hz, 1H), 4.15 (t, *J* = 7.8 Hz, 1H), 3.41−3.33 (m, 2H), 2.46−2.37 (m, 2H), 1.45 (d, *J* = 6.9 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, acetone-d_6) δ = 166.6, 163.8, 162.1, 160.6, 145.7 (d), 142.0, 136.3, 134.7, 131.0, 130.5, 130.3, 129.4, 128.6, 127.9, 127.1, 124.2, 116.0, 115.7, 90.6, 82.4, 48.9, 47.4, 39.4, 36.0, 18.6 ppm; purity (HPLC-MS): 99% t_R: 10.62 min (method: gradient of 10% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{27}H_{26}FN_3O_3$+ H^+ [M + H^+]: 460.20310; found: 460.20133.

3-(3-(1-Hydroxyureido)but-1-yn-1-yl)-N-(3-phenyl-3-(4-(trifluoromethyl)phenyl)propyl)benzamide ***7v***. Procedure F; 155 mg (0.30 mmol) **15m**, 43 mg (0.34 mmol) **8a**, 2 mg (0.006 mmol) $Pd(ACN)_2Cl_2$, 2 mg (0.01 mmol) CuI, 3 mg (0.01 mmol) PPh_3, 0.1 mL (0.37 mmol, 1.2 equiv) DIPA, 8 mL ethyl acetate, 38 h, purification with column chromatography (hexane/acetone 2:3), further purification with preparative HPLC (linear gradient from 10% ACN to 90% within 10 min, 90% ACN for 6 min); white solid (64 mg, 0.13 mmol, 41%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 8.72 (s, 1H), 7.92−7.80 (m, 3H), 7.66−7.19 (m, 11H), 6.14 (s, 2H), 5.28 (q, *J* = 7.0 Hz, 1H), 4.27 (t, *J* = 7.8 Hz, 1H), 3.43−3.35 (m, 2H), 2.53−2.44 (m, 2H), 1.45 (d, *J* = 7.0 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, acetone-d_6) δ = 166.6, 162.1, 150.6, 144.9, 136.2, 134.7, 131.0, 129.5, 129.4, 129.3, 128.7, 127.8, 127.4, 126.2 (q), 124.2, 90.6, 82.3, 47.3, 39.3, 35.6, 18.6 ppm; purity (HPLC-MS): 98% t_R: 11.40 min (method: gradient of 10% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{28}H_{26}F_3N_3O_3$+ H^+ [M + H^+]: 510.19990; found: 510.119834.

3-(3-(1-Hydroxyureido)but-1-yn-1-yl)-N-(3-phenyl-3-(4-(trifluoromethoxy)phenyl)propyl)benzamide ***7w***. Procedure F; 119 mg (0.23 mmol) **15n**, 32 mg (0.25 mmol) **8a**, 1 mg (0.004 mmol) $Pd(ACN)_2Cl_2$, 4 mg (0.02 mmol, 0.09 equiv) CuI, 4 mg (0.01 mmol, 0.06 equiv) PPh_3, 0.1 mL (0.27 mmol, 1.2 equiv) DIPA, 8 mL ethyl acetate, 38 h, purification with column chromatography (hexane/ethyl acetate 1:4), further purification with preparative HPLC (linear gradient from 5% ACN to 90% within 10 min, 90% ACN for 6 min); white solid (30 mg, 0.06 mmol, 25%); ^{1}H-NMR (500 MHz, acetone-d_6) δ = 8.72 (s,1H), 8.08−7.78 (m, 3H), 7.54−7.17 (m, 11H), 6.13 (s, 2H), 5.27 (q, *J* = 7.0 Hz, 1H), 4.21 (t, *J* = 7.8 Hz, 1H), 3.40−3.35 (m, 2H), 2.48−2.42 (m, 2H), 1.45 (d, *J* = 7.0 Hz, 3H) ppm; ^{13}C-NMR (125 MHz, acetone-d_6) δ = 166.6, 162.1, 148.3 (q), 145.4, 145.4, 136.3, 134.8, 131.1, 130.4, 129.6, 129.4, 128.8, 127.9, 124.3, 122.0, 90.7, 82.4, 49.1, 47.4, 39.4, 35.9, 18.7 ppm; purity (HPLC-MS): 97% t_R: 8.41 min (method: gradient of 20% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{28}H_{26}F_3N_3O_4$ + Na^+ [M + Na^+]: 548.17676; found: 548.17552.

N-(3-(4-Chlorophenyl)-3-phenylpropyl)-3-(3-(1-hydroxyureido)-but-1-yn-1-yl)benzamide ***7x***. Procedure F; 108 mg (0.23 mmol) **15o**, 32 mg (0.25 mmol) **8a**, 1 mg (0.01 mmol) $Pd(ACN)_2Cl_2$, 4 mg (0.02 mmol, 0.09 equiv) CuI, 4 mg (0.01 mmol, 0.06 equiv) PPh_3, 0.1 mL (0.27 mmol, 1.2 equiv) DIPA, 8 mL ethyl acetate, 38 h, purification with column chromatography (hexane/ethyl acetate 1:4), further purification with preparative HPLC (linear gradient from 5% ACN to 90% within 10 min, 90% ACN for 6 min); white solid (19 mg, 0.04 mmol, 18%); ^{1}H-NMR (500 MHz, acetone-d_6) δ = 8.72 (s, 1H), 8.09−7.73 (m, 3H), 7.52 (td, *J* = 1.3, 7.6 Hz, 1H), 7.48−7.22 (m, 9H), 7.21−7.16 (m, 1H), 6.14 (s, 2H), 5.27 (q, *J* = 7.0 Hz, 1H), 4.16 (t, *J* = 7.8 Hz, 1H), 3.37 (q, *J* = 6.7 Hz, 2H), 2.42 (q, *J* = 7.4 Hz, 2H), 1.45 (d, *J* = 7.0 Hz, 3H) ppm; ^{13}C-NMR (125 MHz, acetone-d_6) δ = 166.5, 162.0, 145.4, 144.9, 136.2, 134.7, 132.3, 131.0, 130.4, 129.4, 129.3, 129.2, 128.6, 127.8, 127.2, 124.2, 90.6, 82.3, 49.0, 47.3, 39.3, 35.8, 18.6 ppm; purity (HPLC-MS): 98% t_R: 11.21 min (method: gradient of 10% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{27}H_{26}ClN_3O_3$ + H^+ [M + H^+]: 476.17355; found: 476.17231.

(R)-3-(3-(1-Hydroxyureido)but-1-yn-1-yl)-N-(3-(4-(methylsulfonamido)phenyl)-3-phenylpropyl)benzamide ***7y***. Procedure F; 108 mg (0.20 mmol) **15r**, 29 mg (0.22 mmol) **8d**, 1 mg (0.004 mmol) $Pd(ACN)_2Cl_2$, 2 mg (0.01 mmol) CuI, 2 mg (0.01 mmol) PPh_3, 0.3 mL (2.43 mmol, 12 equiv) DIPA, 8 mL ethyl acetate, 40 h, purification with column chromatography (ethyl acetate 100%), further purification with preparative HPLC (5% ACN for 2 min, linear gradient from 5% ACN to 90% within 14 min, 90% ACN for 6 min); white solid (40 mg, 0.08 mmol, 37%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 8.62 (d, *J* = 55.1 Hz, 1H), 7.84−7.80 (m, 3H), 7.53−7.17 (m, 12H), 6.16 (bs, 2H), 5.28 (q, *J* = 7.0 Hz, 1H), 4.12 (t, *J* = 7.8 Hz, 1H), 3.43−3.35 (m, 2H), 2.94 (s, 3H), 2.47−2.38 (m, 2H), 1.46 (d, *J* = 7.0 Hz, 3H) ppm; ^{13}C-NMR (125 MHz, acetone-d_6) δ = 166.5, 162.2 (d), 145.9 (d), 142.0, 136.3, 134.7, 131.0 (d), 129.5, 128.6, 127.0, 124.2, 121.5 (d), 90.6, 82.3, 49.2 (d), 47.3, 39.5, 39.3, 35.9, 18.6 ppm; purity (HPLC-MS): 96% t_R: 12.59 min (method: 5% ACN for 2 min, linear gradient from 5% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{28}H_{30}N_4O_5S$+ H^+ [M + H^+]: 535.20097; found: 535.19989.

(R)-3-(3-(1-Hydroxyureido)but-1-yn-1-yl)-N-(3-phenyl-3-(4-sulfamoylphenyl)-propyl)benzamide ***7z***. Procedure F; 73 mg (140 μmol) **15s**, 20 mg (154 μmol) **8d**, 1 mg (3 μmol) $Pd(ACN)_2Cl_2$, 1 mg (3 μmol, 0.02 equiv) CuI, 2 mg (5 μmol) PPh_3, 0.1 mL (1680 μmol, 12 equiv) DIPA, 5 mL ethyl acetate, 36 h, purification with column chromatography (hexane/acetone 1:2−1:4), further purification with preparative HPLC (5% ACN for 2 min, linear gradient from 5% ACN to 90% within 14 min, 90% ACN for 6 min); white solid (35 mg, 67 μmol, 47%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 8.70 (s, 1H), 7.99−7.77 (m, 5H), 7.58−7.15 (m, 9H), 6.49 (s, 2H), 6.15 (s, 2H), 5.27 (q, *J* = 7.0 Hz, 1H), 4.25 (t, *J* = 7.8 Hz, 1H), 3.43−3.34 (m, 2H), 2.52−2.43 (m, 2H), 1.45 (d, *J* = 7.0 Hz, 3H) ppm; ^{13}C-NMR (125 MHz, acetone-d_6) δ = 161.2, 149.3, 144.1, 142.1, 135.3, 133.8, 130.1, 128.6, 128.8, 128.3, 127.8, 126.9, 126.4, 126.3, 123.3, 89.7, 81.4, 48.5, 46.4, 38.4, 34.6, 17.7 ppm; purity (HPLC-MS): 97% t_R: 12.14 min (method: 5% ACN for 2 min, linear gradient from 5% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{27}H_{28}N_4O_5S$+ H^+ [M + H^+]: 521.18532; found: 521.18435.

(R)-N-(3-(3,4-Dichlorophenyl)-3-phenylpropyl)-3-(3-(1-hydroxyureido)but-1-yn-1-yl)benzamide ***7aa***. Procedure F; 0.15 g (0.29 mmol) **15p**, 41 mg (0.32 mmol) **8d**, 3 mg (0.01 mmol, 0.04 equiv) $Pd(ACN)_2Cl_2$, 2 mg (0.01 mmol) CuI, 6 mg (0.02 mmol, 0.08 equiv) PPh_3, 0.1 mL (0.35 mmol, 1.2 equiv) DIPA, 8 mL ethyl acetate, 38 h, purification with column chromatography (hexane/acetone 1:1−2:3), further purification with preparative HPLC (5% ACN for 2 min, linear gradient from 5% ACN to 90% within 14 min, 90% ACN for 6 min); white solid (80 mg, 0.16 mmol, 53%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 8.75 (s, 1H), 7.92−7.79 (m, 3H), 7.56−7.19 (m, 10H), 6.16 (s, 2H), 5.28 (q, *J* = 7.0 Hz, 1H), 4.19 (t, *J* = 7.8 Hz, 1H), 3.43−3.34 (m, 2H), 2.49−2.39 (m, 2H), 1.45 (d, *J* = 7.0 Hz, 3H) ppm; ^{13}C-NMR (125 MHz, acetone-d_6) δ = 165.7, 161.2, 161.2146.2, 143.9, 135.3, 133.9, 133.7, 131.7, 130.5, 130.1, 129.9, 129.4, 128.7, 128.5, 128.0, 127.8, 127.0, 126.6, 123.3, 89.8, 81.5, 47.9, 46.4, 38.2, 34.6, 17.7 ppm; purity (HPLC-MS): 96% t_R: 14.84 min (method: 5% ACN for 2 min, linear gradient from 5% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{27}H_{25}Cl_2N_3O_3$+ H^+ [M + H^+]: 510.13457; found: 510.13361.

(R)-N-(3-(4-Fluorophenyl)-3-(4-(trifluoromethyl)phenyl)propyl)-3-(3-(1-hydroxyureido)but-1-yn-1-yl)benzamide ***7ab***. Procedure F; 64 mg (121 μmol) **15t**, 17 mg (133 μmol) **8d**, 1 mg (2 μmol)

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$Pd(ACN)_2Cl_2$, 1 mg (5 μmol) CuI, 1 mg (4 μmol) PPh_3, 0.2 mL (1450 μmol, 12 equiv) DIPA, 8 mL ethyl acetate, 65 h, purification with column chromatography (hexane/acetone 2:3), further purification with preparative HPLC (5% ACN for 2 min, linear gradient from 5% ACN to 90% within 14 min, 90% ACN for 6 min); white solid (35 mg, 66 μmol, 55%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 8.65 (s, 1H), 7.91−7.79 (m, 3H), 7.67−7.50 (m, 5H), 7.46−7.39 (m, 3H), 7.14−7.03 (m, 2H), 6.11 (bs, 2H), 5.27 (q, *J* = 7.0 Hz, 1H), 4.31 (t, *J* = 7.8 Hz, 1H), 3.42−3.35 (m, 2H), 2.51−2.43 (m, 2H), 1.45 (d, *J* = 7.0 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, acetone-d_6) δ = 166.5, 163.3, 162.0, 161.4, 150.4, 141.0 (d), 136.2, 134.7, 131.0, 130.6 (d), 129.4, 127.8, 126.3 (q), 124.2, 116.2, 116.0, 90.7, 82.3, 48.5, 47.3, 39.1, 35.7, 18.6 ppm; purity (HPLC-MS): 95% t_R: 15.03 min (method: 5% ACN for 2 min, linear gradient from 5% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{28}H_{25}F_4N_3O_3$ + H^+ [M + H^+]: 528.19048; found: 528.18925.

(R)-N-(3-(4-Fluorophenyl)-3-(4-(trifluoromethoxy)phenyl)-propyl)-3-(3-(1-hydroxyureido)but-1-yn-1-yl)benzamide ***7ac***. Procedure F; 150 mg (276 μmol) **15u**, 39 mg (304 μmol) **8d**, 1 mg (5 μmol) $Pd(ACN)_2Cl_2$, 1 mg (5 μmol, 0.02 equiv) CuI, 3 mg (11 μmol) PPh_3, 0.1 mL (0.33 mmol, 1.2 equiv) DIPA, 8 mL ethyl acetate, 38 h, purification with column chromatography (hexane/ethyl acetate 1:4), further purification with preparative HPLC (5% ACN for 2 min, linear gradient from 5% ACN to 90% within 14 min, 90% ACN for 6 min); white solid (25 mg, 46 μmol, 17%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 8.67 (bs, 1H), 8.03−7.75 (m, 3H), 7.55−7.35 (m, 6H), 7.31−7.18 (m, 2H), 7.10−7.03 (m, 2H), 6.12 (bs, 2H), 5.27 (q, *J* = 7.0 Hz, 1H), 4.24 (t, *J* = 7.8 Hz, 1H), 3.41−3.33 (m, 2H), 2.48−2.39 (m, 2H), 1.45 (d, *J* = 7.0 Hz, 3H) ppm; ^{13}C-NMR (125 MHz, acetone-d_6) δ = 166.6, 163.3, 162.0, 161.3, 148.3 (q), 145.2, 141.3 (d), 136.2, 134.7, 130.5, 130.4, 130.3, 129.4, 127.8, 121.9, 115.9, 90.6, 82.3, 48.1, 47.3, 39.2, 36.0, 18.6 ppm; purity (HPLC-MS): 97% t_R: 15.23 min (method: 5% ACN for 2 min, linear gradient from 5% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{28}H_{25}F_4N_3O_4$ + H^+ [M + H^+]: 544.18540; found: 544.18424.

(R)-N-(3,3-Bis(4-fluorophenyl)propyl)-3-(3-(1-hydroxyureido)but-1-yn-1-yl)benzamide ***7ad***. Procedure F; 177 mg (371 μmol) **15v**, 52 mg (408 μmol) **8d**, 4 mg (12 μmol, 0.04 equiv) $Pd(ACN)_2Cl_2$, 3 mg (15 μmol) CuI, 8 mg (30 μmol, 0.08 equiv) PPh_3, 0.3 mL (0.33 mmol, 5 equiv) DIPA, 8 mL ethyl acetate, 32 h, purification with column chromatography (hexane/acetone 1:1), further purification with preparative HPLC (5% ACN for 2 min, linear gradient from 5% ACN to 90% within 14 min, 90% ACN for 6 min); white solid (60 mg, 126 μmol, 43%); ^{1}H-NMR (400 MHz, acetone-d_6) δ = 8.71 (bs, 1H), 7.93−7.80 (m, 3H), 7.52 (td, *J* = 1.4, 7.7 Hz, 1H), 7.44−7.36 (m, 5H), 7.12−7.01 (m, 4H), 6.13 (bs, 2H), 5.29 (q, *J* = 7.0 Hz, 1H), 4.19 (t, *J* = 7.8 Hz, 1H), 3.39−3.34 (m, 2H), 2.45−2.38 (m, 2H), 1.45 (d, *J* = 6.3 Hz, 3H) ppm; ^{13C}-NMR (100 MHz, acetone-d_6) δ = 166.6, 163.5, 162.1, 161.1, 141.8 (d), 136.3, 134.7, 131.0, 130.4, 130.3, 129.4, 127.8, 124.3, 116.0, 115.8, 90.6, 82.4, 48.0, 47.4, 39.3, 36.2, 18.6 ppm; purity (HPLC-MS): 96% t_R: 14.28 min (method: 5% ACN for 2 min, linear gradient from 5% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{27}H_{25}F_2N_3O_3$ + Na^+ [M + Na^+]: 500.17457; found: 500.17562.

(R)-N-(2-(Diphenylamino)ethyl)-3-(3-(1-hydroxyureido)but-1-yn-1-yl)benzamide ***7ae***. Procedure F; 90 mg (203 μmol) **15x**, 29 mg (223 μmol) **8d**, 2 mg (8 μmol, 0.04 equiv) $Pd(ACN)_2Cl_2$, 2 mg (8 μmol) CuI, 4 mg (16 μmol, 0.08 equiv) PPh_3, 0.1 mL (1015 μmol, 5 equiv) DIPA, 8 mL ethyl acetate, 32 h, purification with column chromatography (hexane/acetone 1:1), further purification with preparative HPLC (5% ACN for 2 min, linear gradient from 5% ACN to 90% within 10 min, 90% ACN for 6 min); white solid (38 mg, 86 μmol, 42%); ^{1}H-NMR (500 MHz, acetone-d_6) δ = 8.71 (s, 1H), 8.03 (t, *J* = 5.8 Hz, 1H), 7.85−7.80 (m, 2H), 7.53 (td, *J* = 1.4, 7.6 Hz, 1H), 7.42 (t, *J* = 7.9 Hz, 1H), 7.31−7.24 (m, 4H), 7.12−7.06 (m, 4H), 6.97−6.91 (m, 2H), 6.13 (s, 2H), 5.28 (q, *J* = 7.0 Hz, 1H), 4.02−3.97 (m, 2H), 3.71−3.64 (m, 2H), 1.46 (d, *J* = 7.2 Hz, 3H) ppm; ^{13}C-NMR (100 MHz, acetone-d_6) δ = 167.0, 162.1, 148.8, 136.0, 134.8, 131.0, 130.2, 129.4, 127.8, 124.3, 122.2, 121.6, 90.7, 82.3, 51.7, 47.3, 38.4, 18.6 ppm; purity (HPLC-MS): 98% t_R: 14.52 min (method: 5% ACN for 2 min, linear gradient from 5% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{26}H_{26}N_4O_3$ + H^+ [M + H^+]: 443.20777; found: 443.20728.

N-((R)-3-(4-Fluorophenyl)-3-(4-(trifluoromethyl)phenyl)propyl)-3-((R)-3-(1-hydroxyureido)but-1-yn-1-yl)benzamide ***7af***. Procedure F; 55 mg (104 μmol) **10u**, 15 mg (114 μmol) **8d**, 1 mg (4 μmol, 0.04 equiv) $Pd(ACN)_2Cl_2$, 1 mg (4 μmol) CuI, 2 mg (8 μmol) PPh_3, 0.3 mL (0.52 mmol, 5 equiv) DIPA, 8 mL ethyl acetate, 32 h, purification with column chromatography (hexane/acetone 1:1), further purification with preparative HPLC (5% ACN for 2 min, linear gradient from 5% ACN to 90% within 14 min, 90% ACN for 6 min); white solid (35 mg, 66 μmol, 64%); ^{1}H-NMR (400 MHz, acetone-d_6) δ = 8.64 (bs, 1H), 7.93−7.80 (m, 3H), 7.66−7.58 (m, 4H), 7.52 (td, *J* = 1.3, 7.8 Hz, 1H), 7.46−7.38 (m, 3H), 7.11−7.03 (m, 2H), 6.09 (bs, 2H), 5.27 (q, *J* = 7.0 Hz, 1H), 4.31 (t, *J* = 7.9 Hz, 1H), 3.41−3.35 (m, 2H), 2.51−2.43 (m, 2H), 1.45 (d, *J* = 7.0 Hz, 3H) ppm; ^{13}C-NMR (100 MHz, acetone-d_6) δ = 166.6, 162.0, 161.5, 150.5, 141.0, 134.8, 131.0, 130.6, 130.5, 129.4, 127.8, 126.3 (q), 124.2, 116.2, 116.0, 90.7, 82.3, 48.6, 47.4, 39.2, 35.8, 18.6 ppm; purity (HPLC-MS): 99% t_R: 15.05 min (method: 5% ACN for 2 min, linear gradient from 5% ACN to 90% within 10 min, 90% ACN for 6 min); HRMS (MALDI): *m/z* calculated $C_{28}H_{25}F_4N_3O_3$ + H^+ [M + H^+]: 528.19048; found: 528.18885.

3-(4-(Trifluoromethyl)phenyl)acrylamide ***23a***. 2.14 g of (9.92 mmol, 1 equiv) **22a** (3-(4-(trifluoromethyl)phenyl)acrylic acid) was dissolved in 15 mL of THF, and 2.3 mL of (16.40 mmol, 1.7 equiv) NEt_3 was added. The mixture was cooled to −15 °C, and 1.7 mL of (16.40 mmol, 1.7 equiv) ethyl chloroformate, diluted in 4 mL THF, was added slowly. The suspension was stirred for 1 h at −15 °C. Additionally, 49 mL of (406.27 mmol, 32%, 41 equiv) NH_4OH was added and the reaction was stirred for 18 h at −15 °C to rt. The suspension was extracted with DCM (3×), and the combined organic phase was washed with saturated aqueous $NaHCO_3$ solution (3×) and brine (1×). After drying over $MgSO_4$ and filtration, the solvent was evaporated under reduced pressure. The solid was recrystallized with ethyl acetate yielding white crystals (1.43 g, 6.66 mmol, 67%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 7.92−7.70 (m, 4H), 7.60 (d, *J* = 15.8 Hz, 1H), 7.08 (s, 1H), 6.86 (d, *J* = 16.7 Hz, 1H), 6.59 (s, 1H) ppm; MS (ESI) *m/z*: 214.85 [M + H^+].

3-(4-(Fluoro)phenyl)acrylamide ***23b***. 2.02 g of (12.15 mmol, 1 equiv) **22b** (3-(4-(fluoro)phenyl)acrylic acid) was dissolved in 25 mL of THF, and 2.8 mL of (20.04 mmol, 1.7 equiv) NEt_3 was added and cooled to −15 °C. Ethyl chloroformate (2.0 mL, 20.04 mmol, 1.7 equiv), diluted in 7 mL THF, was added slowly. The mixture was stirred for 1 h at −15 °C. Additionally, 60 mL of (498.48 mmol, 32%, 41 equiv) NH_4OH was added and the reaction was stirred for 18 h at −15 °C to rt. The suspension was extracted with DCM (3×), and the combined organic phase was washed with saturated aqueous $NaHCO_3$ solution (3×) and brine (1×). After drying over $MgSO_4$ and filtration, the solvent was evaporated under reduced pressure. The solid was recrystallized with ethyl acetate yielding white flakes (1.42 g, 8.61 mmol, 71%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 7.55−7.47 (m, 2H), 7.38 (d, *J* = 15.6 Hz, 1H), 7.08−6.99 (m, 2H), 6.80 (bs, 1H), 6.53 (d, *J* = 16.5 Hz, 1H), 6.27 (bs, 1H) ppm; MS (ESI) *m/z*: 165.60 [M + H^+].

General Procedure for Heck Coupling (Compounds 24a−j, Procedure G). Cinnamamide derivatives **23a−c** (1 equiv) were suspended in triethylamine. For solubility reasons, an organic solvent may be added. The iodoaryl derivative (1.5 equiv) and 0.05 equiv of $Pd(OAc)_2$ were added, and the reaction mixture was heated to 100 °C for 16−48 h. All solvent components were evaporated, and the brown oil was pre-adsorbed on silica gel. Column chromatography yielded a white solid.

3-(4-Fluorophenyl)-3-phenylacrylamide ***24a***. Procedure G; 0.30 g (2.04 mmol) cinnamamide **23c**, 0.68 g (3.06 mmol) 1-fluoro-4-iodobenzene, 0.02 g (0.10 mmol) $Pd(OAc)_2$, 5 mL NEt_3; eluent of column chromatography hexane/acetone 1:1; white solid (0.32 g, 1.34 mmol, 66%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 7.48−7.17

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(m, 7H), 7.15−7.09 (m, 2H), 6.91−6.17 (m, 3H) ppm; MS (ESI) *m/z*: 241.95 [M + H$^+$].

3-Phenyl-3-(4-(trifluoromethyl)phenyl)acrylamide ***24b***. Procedure G; 0.30 g (2.04 mmol) cinnamamide **23c**, 0.85 g (3.06 mmol) 1-iodo-4-(trifluoromethyl)benzene, 0.02 g (0.10 mmol) $Pd(OAc)_2$, 5 mL NEt_3, eluent of column chromatography hexane/acetone 1:1; white solid (0.34 g, 1.17 mmol, 57%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 7.75 (d, J = 8.3 Hz, 2H), 7.57−7.10 (m, 7H), 6.71−6.48 (m, 2H), 6.37−6.32 (m, 1H) ppm; MS (ESI) *m/z*: 291.90 [M + H$^+$].

3-Phenyl-3-(4-(trifluoromethoxy)phenyl)acrylamide ***24c***. Procedure G; 70 mg (0.48 mmol) cinnamamide **23c**, 0.1 mL (0.71 mmol) 1-iodo-4-(trifluoromethoxy)benzene, 5 mg (24 μmol) $Pd(OAc)_2$, 1 mL NEt_3, eluent of column chromatography hexane/acetone 4:1; white solid (0.08 g, 0.25 mmol, 53%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 7.59−7.08 (m, 10H), 7.00 (bs, 1H), 6.45−6.44 (m, 1H) ppm; MS (ESI) *m/z*: 307.85 [M + H$^+$].

3-(4-Chlorophenyl)-3-Phenylacrylamide ***24d***. Procedure G; 0.20 g (1.36 mmol) cinnamamide **23c**, 0.50 g (2.08 mmol) 1-chloro-4-iodobenzene, 0.03 g (0.17 mmol) $Pd(OAc)_2$, 5 mL NEt_3; eluent of column chromatography hexane/acetone 2:1; white solid (0.24 g, 0.92 mmol, 68%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 7.40−7.23 (m, 9H), 6.49−6.26 (m, 3H) ppm; MS (ESI) *m/z*: 257.90 [M + H$^+$].

3-(4-Aminophenyl)-3-phenylacrylamide ***24e***. Procedure G; 1.30 g (8.80 mmol) cinnamamide **23c**, 2.95 g (13.20 mmol) 4-iodoaniline, 0.10 g (0.44 mmol) $Pd(OAc)_2$, 15 mL NEt_3; eluent of column chromatography hexane/acetone 1:3; brown solid (0.98 g, 4.11 mmol, 47%); ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.34−7.29 (m, 3H), 7.11−7.08 (m, 2H), 7.00 (bs, 1H), 6.89−6.83 (m, 2H), 6.67 (bs, 1H), 6.51−6.47 (m, 2H), 6.22 (s, 0.8 H), 6.06 (s, 0.2H), 5.38 (bs, 2H) ppm; MS (ESI) *m/z*: 239.13 [M + H$^+$].

3-Phenyl-3-(4-sulfamoylphenyl)acrylamide ***24f***. Procedure G; 0.35 g (2.36 mmol) cinnamamide **23c**, 1.00 g (3.54 mmol) 4-iodobenzenesulfonamide, 0.03 g (0.12 mmol) $Pd(OAc)_2$, 5 mL NEt_3; eluent of column chromatography hexane/acetone 2:3; white solid (0.35 g, 1.16 mmol, 49%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 7.75−7.71 (m, 2H), 7.34−7.23 (m, 5H), 7.15−7.09 (m, 2H), 6.83−6.30 (m, 5H) ppm; MS (ESI) *m/z*: 302.85 [M + H$^+$].

3-(3,4-Dichlorophenyl)-3-phenylacrylamide ***24g***. Procedure G; 1.50 g (10.20 mmol) cinnamamide **23c**, 4.17 g (15.30 mmol) 1,2-dichloro-4-iodobenzene, 0.12 g (0.51 mmol) $Pd(OAc)_2$, 20 mL NEt_3; eluent of column chromatography hexane/acetone 1:1−2:3; white solid (1.32 g, 4.50 mmol, 44%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 7.58−7.54 (m, 1H), 7.46−7.36 (m, 4H), 7.31−7.21 (m, 3H), 6.56−6.54 (m, 2H), 6.31 (bs, 1H) ppm; MS (ESI) *m/z*: 293.45 [M + 2H$^+$].

3-(4-Fluorophenyl)-3-(4-(trifluoromethyl)phenyl)acrylamide ***24h***. Procedure G, 0.81 g (3.77 mmol) **23a**; 1.23 g (5.65 mmol) 1-fluoro-4-iodobenzene, 0.04 g (0.19 mmol) $Pd(OAc)_2$, 35 mL NEt_3, 12 mL THF, eluent of column chromatography hexane/acetone 1:1−2:3; white solid (0.79 g, 2.56 mmol, 68%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 7.73−7.68 (m, 2H), 7.52−7.27 (m, 4H), 7.18−7.11 (m, 2H), 6.87−6.79 (m, 1H), 6.58−6.57 (m, 1H), 6.31 (bs, 1H) ppm; MS (ESI) *m/z*: 309.65 [M + H$^+$].

3-(4-Fluorophenyl)-3-(4-(trifluoromethoxy)phenyl)acrylamide ***24i***. Procedure G, 0.83 g (5.03 mmol) **23b**, 1.2 mL (7.54 mmol) 1-iodo-4-(trifluoromethoxy)benzene, 0.92 g (4.02 mmol, 0.08 equiv) $Pd(OAc)_2$, 20 mL NEt_3, 9 mL THF, eluent of column chromatography hexane/acetone 2:1−2:3; white solid (1.30 g, 4.01 mmol, 79%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 7.32−7.25 (m, 2H), 7.20−7.12 (m, 4H), 7.03−6.97 (m, 2H), 6.56 (s, 1H), 6.37 (s, 1H), 6.20 (s, 1H) ppm; MS (ESI) *m/z*: 325.75 [M + H$^+$].

3,3-Bis(4-fluorophenyl)acrylamide ***24j***. Procedure G, 0.70 g (4.24 mmol) **23b**, 0.7 mL (6.36 mmol) 1-fluoro-4-iodobenzene, 0.49 g (0.21 mmol) $Pd(OAc)_2$, 20 mL NEt_3, 9 mL THF, eluent of column chromatography hexane/acetone 2:1−2:3; white solid (0.52 g, 2.00 mmol, 47%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 7.37−7.22 (m, 4H), 7.18−7.08 (m, 4H), 6.62 (bs, 1H), 6.44 (s, 1H), 6.28 (bs, 1H) ppm; MS (ESI) *m/z*: 260.15 [M + H$^+$].

General Procedure for Hydrogenation of 25a-j (Procedure H). The acrylamide derivatives **25a−j** were dissolved in methanol or ethanol, and 10 wt % Pd/C or $Pd(OH)_2$ were added. The suspension was set under vacuum and flushed with hydrogen (either in an autoclave or with a balloon). The reaction was stirred for 16−48 h at room temperature. After filtration over diatomaceous earth, the solvent was evaporated, and the yielded oil was used without further purification.

3-(4-Fluorophenyl)-3-phenylpropanamide ***25a***. Procedure H; 0.31 g (1.28 mmol) **24a**, 0.03 g Pd/C, 20 mL MeOH, 40 h, 3.5 bar hydrogen, transparent oil (0.31 g, 1.27 mmol, 99%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 7.47−7.13 (m, 7H), 7.05−6.98 (m, 2H), 6.76 (s, 1H), 6.11 (s, 1H), 4.60 (t, J = 7.8 Hz, 1H), 2.94 (d, J = 7.8 Hz, 2H) ppm; MS (ESI) *m/z*: 243.95 [M + H$^+$].

3-Phenyl-3-(4-(trifluoromethyl)phenyl)propenamide ***25b***. Procedure H; 0.32 g (1.11 mmol) **24b**, 0.03 g Pd/C, 20 mL MeOH, 40 h, 3.1 bar hydrogen, transparent oil (0.32 g, 1.09 mmol, 98%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 7.67−7.49 (m, 4H), 7.37−7.12 (m, 5H), 6.81 (s, 1H), 6.14 (s, 1H), 4.70 (t, J = 7.8 Hz, 1H), 3.01 (dd, J = 4.5, 7.8 Hz, 2H) ppm; MS (ESI) *m/z*: 293.28 [M + H$^+$].

3-Phenyl-3-(4-(trifluoromethoxy)phenyl)propanamide ***25c***. Procedure H; 0.26 g (0.84 mmol) **24c**, 0.03 g Pd/C, 20 mL MeOH, 40 h, 3.2 bar hydrogen, transparent oil (0.24 g, 0.77 mmol, 92%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 7.45−7.19 (m, 9H), 6.79 (bs, 1H), 6.11 (bs, 1H), 4.65 (t, J = 7.8 Hz, 1H), 2.97 (dd, J = 1.7, 7.8 Hz, 2H) ppm; MS (ESI) *m/z*: 309.90 [M + H$^+$].

3-(4-Chlorophenyl)-3-phenylpropanamide ***25d***. Procedure H; 0.20 g (0.78 mmol) **24d**, 0.01 g $Pd(OH)_2$ on charcoal, 15 mL EtOH, 18 h, hydrogen atmosphere via a balloon, transparent oil (0.19 g, 0.71 mmol, 92%); ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 7.65−7.10 (m, 11H), 4.60 (t, J = 7.9 Hz, 1H), 3.13−2.78 (m, 2H) ppm; MS (ESI) *m/z*: 259.65 [M + H$^+$].

3-(4-Aminophenyl)-3-phenylpropanamide ***25e***. Procedure H; 0.48 g (2.02 mmol) **24e**, 0.05 g Pd/C, 50 mL MeOH, 72 h, 3.8 bar hydrogen, transparent oil (0.48 g, 1.99 mmol, 99%); ^{1}H-NMR (300 MHz, acetone-d_6) δ = 7.33−6.99 (m, 9H), 6.63−6.58 (m, 3H), 6.06 (bs, 1H), 4.51−4.43 (m, 1H), 3.00−2.96 (m, 2H) ppm; MS (ESI) *m/z*: 240.95 [M + H$^+$].

3-Phenyl-3-(4-sulfamoylphenyl)propenamide ***25f***. Procedure H; 0.35 g (1.12 mmol) **24f**, 0.04 g Pd/C, 20 mL MeOH, 43 h hydrogen atmosphere via a balloon, transparent oil (0.26 g, 0.87 mmol, 75%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 7.83−7.74 (m, 2H), 7.52−7.46 (m, 2H), 7.32−7.28 (m, 5H), 6.83 (bs, 1H), 6.49 (s, 2H), 6.16 (bs, 1H), 4.68 (t, J = 7.8 Hz, 1H), 3.01 (dd, J = 5.3, 7.9 Hz, 2H) ppm; MS (ESI) *m/z*: 346.10 [M + ACN + H$^+$].

3-(3,4-Dichlorophenyl)-3-phenylpropanamide ***25g***. Procedure H; 0.10 g (0.39 mmol) **24g**, 0.01 g $Pd(OH)_2$ on charcoal, 6 mL EtOH, 18 h, 3 bar hydrogen, transparent oil (0.10 g, 0.33 mmol, 85%); ^{1}H-NMR (250 MHz, $CDCl_3$) δ = 7.55−7.40 (m, 2H), 7.39−7.12 (m, 6H), 6.79 (bs, 1H), 6.12 (bs, 1H), 4.61 (t, J = 7.5 Hz, 1H), 3.11−2.85 (m, 2H) ppm; MS (ESI) *m/z*: 337.75 [M + ACN + H$^+$].

3-(4-Fluorophenyl)-3-(4-(trifluoromethyl)phenyl)propanamide ***25h***. Procedure H; 0.56 g (1.82 mmol) **24h**, 0.06 g Pd/C, 20 mL MeOH, 18 h; 3.3 bar hydrogen, transparent oil (0.57 g, 1.82 mmol, 100%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 7.77−7.31 (m, 6H), 7.09−7.01 (m, 2H), 6.82 (bs, 1H), 6.14 (bs, 1H), 4.71 (t, J = 7.8 Hz, 1H), 3.03−2.98 (m, 2H) ppm; MS (ESI) *m/z*: 311.85 [M + H$^+$].

3-(4-Fluorophenyl)-3-(4-(trifluoromethoxy)phenyl)propanamide ***25i***. Procedure H; 0.40 g (1.23 mmol) **24i**, 0.04 g Pd/C, 25 mL MeOH, 14 h hydrogen atmosphere via a balloon, transparent oil (0.39 g, 1.19 mmol, 96%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 7.56−7.18 (m, 6H), 7.09−6.99 (m, 2H), 6.79 (bs, 1H), 6.13 (bs, 1H), 4.66 (t, J = 7.8 Hz, 1H), 2.99−2.94 (m, 2H) ppm; MS (ESI) *m/z*: 327.75 [M + H$^+$].

3,3-Bis(4-fluorophenyl)propenamide ***25j***. Procedure H; 0.50 g (1.93 mmol) **24j**, 0.05 g Pd/C, 30 mL MeOH, 43 h hydrogen atmosphere via a balloon, transparent oil (0.37 g, 1.42 mmol, 74%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 7.42−7.20 (m, 4H), 7.07−6.97 (m, 4H), 6.78 (bs, 1H), 6.15 (bs, 1H), 4.61 (t, J = 7.8 Hz, 1H), 2.93 (d, J = 8.3 Hz, 2H) ppm; MS (ESI) *m/z*: 261.85 [M + H$^+$].

General Procedure for Reduction of Primary Amides 25a−j to the Corresponding Amines 10i,k−s (Procedure I). Amide derivatives **25a−j** (1 equiv) were dissolved in dry THF and cooled to

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0 °C. $LiAlH_4$ solution (1.5−2.5 equiv) in THF was added slowly. The mixture was heated under reflux conditions for 2−5 h. After cooling to room temperature, the hydride was quenched with acetone or ethyl acetate and stirred with saturated aqueous potassium sodium tartrate solution for at least 30 min. The phases were separated, and the organic solvent was removed under reduced pressure. The residue was purified with column chromatography to obtain a colorless oil.

3-(4-Fluorophenyl)-3-phenylpropan-1-amine ***10i***. Procedure I; 0.30 g (1.23 mmol) **25a**, 3.1 mL (3.0 mmol, 1 M in THF) $LiAlH_4$ solution, 5 mL THF, 5 h reflux, eluent of column chromatography hexane/acetone 1:2; 1% NEt_3; yellow oil (0.17 g, 0.72 mmol, 59%); ^{1}H-NMR (250 MHz, $CDCl_3$) δ = 7.23 (s, 7H), 6.92−6.83 (m, 2H), 3.95 (t, J = 7.9 Hz, 1H), 3.05 (t, J = 7.2 Hz, 2H), 1.92 (t, J = 1.3 Hz, 2H) ppm; MS (ESI) m/z: 229.95 [M + H$^+$].

3-Phenyl-3-(4-(trifluoromethyl)phenyl)propan-1-amine ***10k***. Procedure I; 0.31 g (1.06 mmol) **25b**, 2.7 mL (2.65 mmol, 1 M in THF) $LiAlH_4$ solution, 5 mL THF, 5 h reflux, eluent of column chromatography DCM/MeOH 10:1; yellow oil (0.14 g, 0.49 mmol, 46%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 7.65−7.55 (m, 4H), 7.38−7.17 (m, 5H), 4.32 (t, J = 7.4 Hz, 1H), 3.12 (t, J = 6.6 Hz, 2H), 2.39 (q, J = 7.3 Hz, 2H) ppm; MS (ESI) m/z: 279.90 [M + H$^+$].

3-Phenyl-3-(4-(trifluoromethoxy)phenyl)propan-1-amine ***10l***. Procedure I; 0.59 g (1.89 mmol) **25c**, 8.5 mL (8.51 mmol, 1 M in THF) $LiAlH_4$ solution, 5 mL THF, 4 h reflux, eluent of column chromatography DCM/$MeOH_{ammonia}$ 10:0.1−10:1; yellow oil (0.15 g, 0.52 mmol, 28%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 7.48−7.42 (m, 2H), 7.40−7.06 (m, 7H), 4.24 (t, J = 7.7 Hz, 1H), 3.14 (t, J = 7.7 Hz, 2H), 2.35 (q, J = 7.3 Hz, 2H) ppm; MS (ESI) m/z: 296.21 [M + H$^+$].

3-(4-Chlorophenyl)-3-phenylpropan-1-amine ***10m***. Procedure I; 0.19 g (0.71 mmol) **25d**, 1.8 mL (1.78 mmol, 1 M in THF) $LiAlH_4$ solution, 7 mL THF, 6 h reflux, eluent of column chromatography DCM/$MeOH_{ammonia}$ 95:5; yellow oil (0.09 g, 0.37 mmol, 51%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 7.35−7.13 (m, 9H), 4.18 (t, J = 8.0 Hz, 1H), 3.09 (t, J = 8.2 Hz, 2H), 2.38−2.24 (m, 2H) ppm; MS (ESI) m/z: 245.70 [M + H$^+$].

3-(3,4-Dichlorophenyl)-3-phenylpropan-1-amine ***10n***. Procedure I; 1.26 g (4.29 mmol) **25g**, 9.4 mL (9.44 mmol, 1 M in THF) $LiAlH_4$ solution, 10 mL THF, 6 h reflux, eluent of column chromatography DCM/$MeOH_{ammonia}$ 95:5; yellow oil (0.53 g, 1.89 mmol, 44%); ^{1}H-NMR (400 MHz, acetone-d_6) δ = 7.55−7.42 (m, 2H), 7.32−7.28 (m, 6H), 4.22 (t, J = 7.5 Hz, 1H), 3.11 (t, J = 7.5 Hz, 2H), 2.42−2.25 (m, 2H) ppm; MS (ESI) m/z: 322.25 [M + ACN + H$^+$].

4-(3-Amino-1-phenylpropyl)aniline ***10o***. Procedure I; 0.48 g (1.99 mmol) **25e**, 5.0 mL (4.97 mmol, 1 M in THF) $LiAlH_4$ solution, 20 mL THF, 16 h reflux, eluent of column chromatography DCM/$MeOH_{ammonia}$ 98:2−95:5; yellow oil (0.23 g, 0.99 mmol, 50%); ^{1}H-NMR (250 MHz, CD_3CN) δ = 7.42−6.90 (m, 7H), 6.58−6.52 (m, 2H), 4.31−3.69 (m, 3H), 2.55−2.45 (m, 2H), 2.10−2.01 (m, 2H) ppm; MS (ESI) m/z: 227.00 [M + H$^+$].

4-(3-Amino-1-phenylpropyl)benzenesulfonamide ***10p***. Procedure I; 0.53 g (1.74 mmol) **25f**, 7.8 mL (7.81 mmol, 1 M in THF) $LiAlH_4$ solution, 26 mL THF, 5 h reflux, eluent of column chromatography DCM/$MeOH_{ammonia}$ 98:2; yellow oil (0.20 g, 0.67 mmol, 39%); ^{1}H-NMR (250 MHz, acetone-d_6) δ = 7.84−7.77 (m, 2H), 7.54−7.48 (m, 2H), 7.37−7.30 (m, 4H), 7.23−7.14 (m, 1H), 6.47 (s, 2H), 4.29 (t, J = 7.9 Hz, 1H), 3.11 (t, J = 6.9 Hz, 2H), 2.37 (q, J = 7.2 Hz, 2H) ppm; MS (ESI) m/z: 289.10 [M − H$^+$].

3-(4-Fluorophenyl)-3-(4-(trifluoromethyl)phenyl)propan-1-amine ***10q***. Procedure I; 0.39 g (1.24 mmol) **25h**, 3.7 mL (3.72 mmol, 1 M in THF) $LiAlH_4$ solution, 15 mL THF,4 h reflux, eluent of column chromatography DCM/$MeOH_{ammonia}$ 10:0.1−10:1; yellow oil (0.23 g, 0.79 mmol, 64%); ^{1}H-NMR (250 MHz, CD_3CN) δ = 7.66−7.61 (m, 2H), 7.52 (d, J = 8.1 Hz, 2H), 7.40−7.33 (m, 2H), 7.12−7.03 (m, 2H), 4.35 (t, J = 7.9 Hz, 1H), 3.43 (2H, bs), 2.84−2.78 (m, 2H), 2.54−2.45 (m, 2H) ppm; MS (ESI) m/z: 339.00 [M + ACN + H$^+$].

3-(4-Fluorophenyl)-3-(4-(trifluoromethoxy)phenyl)propan-1-amine ***10r***. Procedure I; 0.67 g (2.07 mmol) **25i**, 4.4 mL (4.35 mmol, 1 M in THF) $LiAlH_4$ solution, 5 mL THF, 4 h reflux, eluent of column chromatography DCM/$MeOH_{ammonia}$ 10:0.1−10:1; yellow oil (0.34 g, 1.09 mmol, 52%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 7.44−7.24 (m, 6H), 7.14−7.06 (m, 2H), 4.19 (t, J = 8.2 Hz, 1H), 2.45−2.37 (m, 2H), 2.13−2.02 (m, 2H) ppm; MS (ESI) m/z: 313.90 [M + H$^+$].

3,3-Bis(4-fluorophenyl)propan-1-amine ***10s***. Procedure I; 0.34 g (1.31 mmol) **25j**, 3.9 mL (3.94 mmol, 1 M in THF) $LiAlH_4$ solution, 5 mL THF, 3.5 h reflux, eluent of column chromatography DCM/MeOH 9:1; yellow oil (0.16 g, 0.66 mmol, 50%); ^{1}H-NMR (250 MHz, CD_3CN) δ = 7.34−7.25 (m, 4H), 7.07−7.00 (m, 4H), 4.10 (t, J = 7.5 Hz, 1H), 2.50 (t, J = 7.0 Hz, 2H), 2.13−2.04 (m, 2H) ppm; MS (ESI) m/z: 247.90 [M + H$^+$].

N-(2-Chloroethyl)-N-phenylaniline ***27***. 3.5 mL of (58.5 mmol, 6.5 equiv) chloroacetic acid was diluted in 100 mL of toluene over a time period of 20 min. 1.7 g of (45.0 mmol, 5 equiv) $NaBH_4$ was added portionwise. The suspension stirred for 3 h at room temperature. Additionally, 1.3 mL of (9.0 mmol, 1 equiv) diphenylamine **26** was added and the reaction mixture was stirred for 4 h under reflux conditions. The reaction was allowed to cool to room temperature and was quenched with 2 M aqueous NaOH. The phases were separated, and the organic phase was washed with brine. The organic phase was dried over $MgSO_4$ and filtered, and the solvent was removed under reduced pressure. Column chromatography (hexane/ethyl acetate 15:1) obtained a transparent oil (1.4 g, 6.0 mmol, 67%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 7.33−7.26 (m, 4H), 7.07−6.92 (m, 6H), 4.05 (t, J = 6.8 Hz, 2H), 3.75 (t, J = 6.7 Hz, 2H) ppm; MS (ESI) m/z: 231.85 [M + H$^+$].

2-(2-(Diphenylamino)ethyl)isoindolin-1,3-dione ***28***. 1.39 g of (6 mmol, 1 equiv) **27** was dissolved in 5 mL of DMF, and 1.12 g of (6 mmol, 1 equiv) potassium phthalimide was added. The mixture was heated to 140 °C for 1 h under microwave irradiation. The reaction mixture was diluted with ethyl acetate and washed with water (3×). The aqueous phase was extracted with ethyl acetate, and the combined phases were dried over $MgSO_4$. After filtration and evaporation, the residue was purified with column chromatography (hexane/ethyl acetate 9:1−5:1). A yellow wax was isolated (1.32 g, 3.86 mmol, 64%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 7.77 (s, 4H), 7.24−7.17(m, 4H), 7.00−6.84 (m, 6H), 4.04−3.99 (m, 2H), 3.86−3.81 (m, 2H) ppm; MS (ESI) m/z: 365.05 [M + Na$^+$].

N^1,N^1-Diphenylethan-1,2-diamine ***10t***. 1.22 g of (3.56 mmol, 1 equiv) **28** was dissolved in 10 mL of EtOH. 1.8 mL of (35.60 mmol, 10 equiv) hydrazine hydrate was added, and the reaction was stirred for 16 h under reflux conditions and 20 h at room temperature. The solvent was removed, and the residue was suspended in hexane. The solid was filtered, and the organic solvent was evaporated under reduced pressure. Column chromatography yielded a yellow oil (0.10 g, 0.49 mmol, 14%); ^{1}H-NMR (250 MHz, DMSO-d_6) δ = 7.24−7.22 (m, 6H), 7.01−6.87 (m, 4H), 6.68 (bs, 2H), 3.67 (t, J = 7.1 Hz, 2H), 2.71 (t, J = 7.3 Hz, 2H) ppm; MS (ESI) m/z: 212.90 [M + H$^+$].

(S,E)-4-(tert-Butyl)-3-(3-(4-(trifluoromethyl)phenyl)acryloyl)-oxazolidin-2-one ***30***. To a solution of 0.34 g of (2.32 mmol, 1 equiv) (*S*)-4-tert-butyl-2-oxazolidinone, 1.0 mL of (2.55 mmol, 2.5 M in THF, 1.1 equiv) *n*BuLi was added dropwise at −78 °C. The mixture was stirred for 15 min, and afterward, 0.54 g of (2.32 mmol) **29** ((*E*)-3-(4-(trifluoromethyl)phenyl)acryloyl chloride), dissolved in 4 mL of dry DCM, was added dropwise and stirred for 1 h at −78 °C and 30 min at 0 °C. (**29** was prepared as follows: 0.52 g of (2.32 mmol, 1 equiv) **22a** ((*E*)-3-(4-trifluoromethyl)-phenyl)acrylic acid) was solved in 4 mL of dry THF and cooled with an ice bath. 0.3 mL of (2.78 mmol, 1.2 equiv) oxalyl chloride was added, and the reaction mixture was stirred for 17 h at room temperature. The suspension was evaporated under reduced pressure). The mixture was quenched with saturated aqueous NH_4Cl solution and extracted with ethyl acetate (3×). The combined organic phase was dried over $MgSO_4$ and filtered, and the solvent was evaporated. The residue was purified with column chromatography (hexane/ethyl acetate 9:1, 5:1, 3:1); yellow oil (0.53 g, 1.55 mmol, 67%), ^{1}H-NMR (250 MHz, $CDCl_3$) δ = 8.01 (d, J = 15.7 Hz, 1H), 7.91−7.58 (m, 5H), 4.62−4.55 (m, 1H), 4.38−4.22 (m, 2H), 0.98 (s, 9H) ppm; MS (ESI) m/z: 342.03 [M + H$^+$].

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(S)-4-(tert-Butyl)-3-((R)-3-(4-fluorophenyl)-3-(4-(trifluoromethyl)-phenyl)-propanoyl)oxazolidin-2-one ***31***. 0.32 g of (0.94 mmol, 1 equiv) **30**, 0.26 g of (1.88 mmol, 2 equiv) (4-fluorophenyl)boronic acid, 0.01 g of (0.05 mmol, 0.05 equiv) $Pd(OAc)_2$, and 0.03 g of (0.19 mmol, 0.2 equiv) 2,2′-bipyridine were suspended in MeOH/water (1:3) in a microwave vial. The vial was sealed and heated to 80 °C for 14 h. The suspension was diluted with ethyl acetate and washed with 2 M aqueous NaOH (2×), water (1×), and brine (1×). The organic phase was dried over $MgSO_4$ and filtered, and the solvent was evaporated under reduced pressure. The residue was purified with column chromatography (hexane/ethyl acetate 5:1−3:1); off-white solid (0.25 g, 0.56 mmol, 60%); ^{1}H-NMR (250 MHz, $CDCl_3$) δ = 7.55 (d, J = 8.2 Hz, 2H), 7.40 (d, J = 8.3 Hz, 2H), 7.34−7.08 (m, 2H), 7.02−6.94 (m, 2H), 4.74 (t, J = 7.8 Hz, 1H), 4.38−4.09 (m, 3H), 3.91 (dd, J = 7.8, 16.7 Hz, 1H), 3.60 (dd, J = 7.9, 16.7 Hz, 1H), 0.75 (s, 9H) ppm; MS (ESI) m/z: 436.15 [M - H$^+$].

(R)-3-(4-Fluorophenyl)-3-(4-(trifluoromethyl)phenyl)propan-1-ol ***32***. Under an inert atmosphere, 0.41 g of (0.94 mmol) **31** was dissolved in 5 mL of dry Et_2O and cooled with an ice bath. 0.8 mL of (1.68 mmol, 1.8 equiv) $LiBH_4$ suspension in dry THF was added, and the mixture stirred for 4 h at room temperature. The reaction was quenched with acetone and stirred with saturated aqueous potassium sodium tartrate solution for 30 min. The solution was extracted with ethyl acetate (3×), and the combined organic phases were dried over $MgSO_4$. After filtration and removal of the solvent, the residue was purified with column chromatography (hexane/acetone 7:1−3:1); white solid (0.16 g, 0.53 mmol, 57%); ^{1}H-NMR (250 MHz, $CDCl_3$) δ = 7.56−7.52 (m, 2H), 7.36−7.33 (m, 2H), 7.24−7.16 (m, 2H), 7.02−6.96 (m, 2H), 4.24 (t, J = 7.9 Hz, 1H), 3.60 (t, J = 6.0 Hz, 2H), 2.33−2.26 (m, 2H), 1.55 (bs, 1H) ppm; MS (ESI) m/z: 297.15 [M − H$^+$].

(R)-3-(4-Fluorophenyl)-3-(4-(trifluoromethyl)phenyl)propanal ***33***. Under an inert atmosphere, 0.15 g of (0.51 mmol) **32**, 0.33 g of (0.77 mmol, 1.5 equiv) Dess−Martin periodinane in 10 mL of DCM, and 0.01 mL of water were stirred for 2.5 h at room temperature. The suspension was diluted with DCM and quenched with saturated aqueous $NaHCO_3$ solution/saturated aqueous NaS_2O_3 solution (1:1). The phases were separated, and the aqueous phase was extracted with DCM (3×). The combined organic phase was washed with saturated aqueous $NaHCO_3$ solution (2×), water (2×), and brine (1×). The organic phase was dried over $MgSO_4$, filtered, and evaporated. The purification with column chromatography (hexane/ethyl acetate 5:1−2:1) yielded a transparent oil (0.11 g, 0.37 mmol, 72%), ^{1}H-NMR (250 MHz, $CDCl_3$) δ = 9.76 (t, J = 1.5 Hz, 1H), 7.56 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8.3 Hz, 2H), 7.22−7.13 (m, 2H), 7.06−6.95 (m, 2H), 4.69 (t, J = 7.6 Hz, 1H), 3.19 (dd, J = 1.5, 7.6 Hz, 2H) ppm; MS (ESI) m/z: 295.25 [M − H$^+$].

(R)-3-(4-Fluorophenyl)-3-(4-(trifluoromethyl)phenyl)propan-1-amine ***10u***. A saturated solution of 0.29 g of (3.68 mmol, 10 equiv) dry NH_4OAc in 20 mL of ethanol was prepared. To this solution, 0.11 g of (0.37 mmol) **33** was added and a suspension was formed. 0.49 g of (2.21 mmol, 6 equiv) sodium triacetoxyborohydride and 3 mL of (32% in water, 24.1 mmol, 66 equiv) ammonia were added, and the mixture stirred for 13 h under reflux conditions. The reaction mixture was allowed to cool to room temperature and was diluted with ethyl acetate and saturated aqueous $NaHCO_3$ (1:1). The phases were separated, and the aqueous phase was extracted with ethyl acetate (3×). The combined organic phase was dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure, and the residue was purified via column chromatography (DCM/MeO-$H_{ammonia}$ 9:1). A yellowish oil was obtained (0.05 g, 0.18 mmol, 48%); ^{1}H-NMR (250 MHz, $CDCl_3$) δ = 7.56−7.48 (m, 2H), 7.35−7.29 (m, 2H), 7.21−7.16 (m, 2H), 7.01−6.90 (m, 2H), 4.60 (s, 2H), 4.03−4.03 (m, 1H), 2.88−2.74 (m, 2H), 2.56−2.41 (m, 2H) ppm; MS (ESI) m/z: 298.10 [M + H$^+$].

sEH Expression and Purification. The full length protein (aa1−aa555) was isolated as published previously by Hahn et al.[37] and Lukin et al.[38] The protocols were slightly modified. In brief, sEH was expressed in *E. coli* BL21-(DE3) cells with the ZYP5052 auto-induction media supplemented with kanamycin at 16 °C for 36 h. After lyses, the protein was isolated by nickel affinity chromatography and size exclusion chromatography. The buffer for the size exclusion was 50 mM Tris, 500 mM NaCl, 5% glycerol (HCl) pH 8. If the protein was stored at −80 °C, glycerol was added (final concentration of 25% v/v). Aliquots of the protein were flash-frozen with liquid nitrogen and stored at −80 °C.

The protocol for the sEH hydrolase expression is performed as described previously.[39]

sEH-H Activity Assay with PHOME. To determine the IC_{50} values of the potential inhibitors, a fluorescence assay was adapted from Hahn et al.[37] and Lukin et al.[38] The non-fluorescent substrate PHOME (3-phenyl-cyano(6-methoxy-2-naphthalenyl)methyl ester-2-oxiraneacetic acid) is hydrolyzed to the fluorescent 6-methoxy-2-naphtaldehyde by the sEH hydrolase activity. To monitor the fluorescence (λ_{ex} = 360 nm and λ_{em} = 465 nm), 1 μL of a DMSO dilution series of the compound to test was pipetted into a black flat-bottom 96-well plate. A mix (89 μL) of recombinant human full length sEH (final concentration of 3 nM in each well) in Bis-Tris buffer (pH 7) with 0.1 mg/mL BSA and Triton-X 100 (0.01% (w/v) final concentration) was added. After an incubation time of 30−45 min, 10 μL of substrate in buffer (50 μM final concentration) was added quickly. The fluorescence was monitored for 30−45 min (one point per minute) using a Tecan Infinite F200 Pro plate reader. As a blank, 1 μL of pure DMSO in buffer without protein and, as a positive control, 1 μL of pure DMSO in a protein−buffer mix were used. All experiments were done in triplicate and in three independent measurements. To analyze the inhibitory potential of the tested compounds, the percent inhibition was calculated by referencing the slope (in the linear phase) of the reaction to the slopes of the positive and negative controls. Further fitting was performed with the software GraphPad Prism 7 with a sigmoidal dose response curve fit (variable slope with four parameters).

5-LOX Expression and Purification. For purification of recombinant 5-LOX, the enzyme was expressed in a 1 L culture (*E. coli* BL21-(DE3)) and purified using ATP affinity chromatography with a 5 mL ATP agarose column followed by anion exchange chromatography on a ResourceQ 1 mL IEX column in an ÄKTA Xpress system (GE Healthcare).

5-LOX Activity Assay with Recombinantly Expressed Protein. To determine inhibition of 5-LOX, test compounds or vehicles were diluted in 1 mL of reaction buffer containing 3 μg of 5-LOX, 1 mM EDTA, and 1 mM ATP in PBS. After 15 min of pre-incubation at 4 °C, the samples were pre-warmed for 30 s and the reaction was started with 20 μM AA and 2 mM $CaCl_2$. The reaction was stopped after 10 min at 37 °C with ice-cold methanol (1 mL). Formed 5-LOX products (5-H(p)ETE, all-trans LTB_4 and LTB_4) were analyzed by HPLC/UV. In brief, after solid-phase extraction, prostaglandin B1 (200 ng) was added as an internal standard to the samples as well as HCl (30 μL, 1 N) and PBS (500 μL) and HPLC analysis (as described by Brungs et al.[40] and Steinhilber et al.[41]) of the 5-LOX products was performed. Data was normalized to vehicle control (DMSO), and IC_{50} values with 95% confidence intervals were calculated.

Isolation of Intact PMNL. Leukocyte concentrates were used to freshly isolate human PMNLs according to Werz et al.[42] In brief, PMNLs were immediately isolated by dextran sedimentation and centrifugation on lymphocyte separation medium followed by hypotonic lysis of erythrocytes. Cells (purity of >96 to 97%) were finally resuspended in PBS (pH 7.4) containing 1 mg/mL glucose (PG).

5-LOX Activity Assay in Intact Cells (PMNL). For determination of 5-LOX product formation in intact cells, freshly isolated PMNLs (5×10^6) were resuspended in PG and 1 mM $CaCl_2$, preincubated with test compounds or vehicle control for 15 min at 37 °C, and stimulated by the addition of AA (20 μM) and calcium ionophore A23187 (2.5 μM). The reaction was stopped after 10 min at 37 °C by the addition of methanol (1 mL), and 5-LOX product formation was analyzed by HPLC/UV as described for recombinant protein.

Crystallization of 7w with the sEH-H. The protocol for the crystallization of the sEH-H with **7w** is based on the original

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publication of Xing et al.[43] and was previously published for other sEH-H inhibitors by Lukin et al.[38] as well as Hiesinger et al.[39] The soaking of the sEH-H crystals with **7w** was performed as described in these publications. X-ray diffraction data of a single crystal was collected at the beamline station ID29 at the European Synchrotron Radiation Facility (ESRF), Grenoble, France. All diffraction data was obtained from a single crystal and processed with the XDS Software package.[44]

The structure of sEH-H with **7w** at 2.00 Å resolution was obtained by molecular replacement using the PHENIX software package[45] using a truncated version of the full length sEH structure (PDB: 5ALU[46]) where coordinates for heteroatoms (water and ligands) were excluded from the starting model. After iterative rounds of model building with COOT[47] into the $2F_o-F_c$ electron density map, the model containing the polypeptide chain and the ligand was refined to final values of 0.184 for R_{work} and 0.207 for R_{free}. As the four different stereoisomers were present in the **7w** compound mixture, stereochemical restrains for all four variants were generated using the eLBOW[48] tool of the PHENIX software package and tested in the refinement process. Modeling of the stereoisomers of **7w** into unexplained electron density in the vicinity of the binding site indicated that all four diastereomers can be interpreted by the electron density. The decision on the most likely pose of the **7w** diastereomer has been made on random.

The graphical representations of the model and electron density were made using MOE (Chemical Computing Group, Montreal, Canada). Statistics of data collection and structural refinement are summarized in Table S1 and were generated using the PHENIX table one tool. The coordinates and structure-factor amplitudes of the structure have been deposited in the Protein Data Bank as entry 6YL4.

Determination of the Water Solubility Limit. A dilution series of the compounds in DMSO was prepared. DMSO stocks (1 μL) were pipetted in the corresponding wells. As a blank, 1 μL of pure DMSO was used. DPBS buffer (99 μL, pH 7.4) with 0.01% (w/v) Triton X-100 was added, and the absorption was measured using a Tecan infinite M200 at 650 nm. The values of means were compared to the mean of the blank. If the values differ, the compound is not solved completely and the change in absorption behavior marks the solubility limit.

Metabolic Stability in Rat Liver Microsomes. The determination of metabolic stability was performed as described by Schierle et al.[49]

PK Study. The study was performed by Pharmacelsus GmbH and approved by and conducted in accordance with the regulation of the local animal welfare authorities: Landesamt für Gesundheit und Verbraucherschutz, Abteilung Lebensmittel- und Veterinärwesen, Saarbrücken. A detailed description can be found in the Supporting Information.

EETS/DHET Ratio. The determination of the lipid levels was performed as described by Blöcher et al.[31]

Unilateral Ureteral Obstruction Model. Obstruction of the left ureter was performed in 10 to 12 week-old male C57BL/6 mice as reported previously.[50] Briefly, after a left flank incision, the ureter was exposed and doubly ligated using 6-0 prolene followed by closing of the incision under aseptic conditions in ketamine/xylazine-anesthetized mice (200 mg/10 mg/kg). The contralateral kidney served as a control. The vehicle (20% hydroxy-propyl-β-cyclodextrine/PBS, AppliChem GmbH, Darmstadt, Germany) or **7ad** (3 mg/kg) was intraperitoneally administered to mice daily from day 1 after surgery. Kidneys were analyzed at day 7 after ligation of the left ureter.

Renal Histology and Immunohistochemistry. Paraffin-embedded renal sections (5 μm thick) were deparaffinized and stained either with AZAN trichrome stain or Sirius Red for the detection of interstitial collagen as previously described.[50] All sections were evaluated with an Olympus BX60 microscope (Olympus, Hamburg, Germany) at 200× magnification (additionally using a polarization filter for Sirius Red). Images of the renal cortex were acquired using an Olympus DP70 camera and examined by a blinded observer. Collagen content of the Sirius Red-stained slides was evaluated using ImageJ software version 1.51k (NIH, Bethesda, MD) from 5−8 randomly selected non-overlapping fields avoiding large vessels, by setting a fixed background intensity threshold and calculating unmasked pixels above threshold relative to total pixels. For immunohistochemical staining of F4/80 (macrophage marker), sections were incubated with anti-F4/80 (clone Cl:A3-1; Bio-Rad Laboratories GmbH, München, Germany) overnight at 4 °C. Diaminobenzidine (DAB, Vector-Laboratories, Burlingame, CA) was used for visualization, and counterstaining was performed with Mayer's hematoxylin. Images of the renal cortex were acquired using an Olympus DP70 camera and examined by a blinded observer. The F4/80 positive area was evaluated using the Fuji plugin of ImageJ software version 1.51k of 4−8 randomly selected non-overlapping fields using the color deconvolution for DAB−4hematoxylin. The brown image was converted to an 8 pixel black/white image followed by setting a fixed background intensity threshold and calculating unmasked pixels above the threshold relative to total pixels.

RNA Extraction and Real-Time Quantitative PCR Analysis. Total RNA from homogenized mouse kidneys was isolated using a TRI reagent (Sigma-Aldrich, Steinheim, Germany) as previously described[50] and reverse-transcribed using a RevertAid first-strand cDNA synthesis kit (Thermo Fisher Scientific, Waltham, MA). Real-time quantitative PCR (TaqMan) was performed with an ABI prism 7500 sequence detection system using TaqMan gene expression assays and a real-time quantitative PCR Low Rox Mix (Life Technologies, Darmstadt, Germany). The following TaqMan gene expression assays were used: murine collagen 1a1 (Col1a1), Mm00801666_g1; murine fibronectin-1 (FN-1), Mm01256744_m1; murine tumor necrosis factor α (TNFα) Mm00443258_m1; murine CC-chemokine ligand 2 (Ccl2) Mm00441242_m1, murine Gapdh, 4352339E (Life Technologies, Darmstadt, Germany). The threshold cycle for the gene of interest was normalized to that of Gapdh.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c00561.

Cell viability assay, additional X-ray data, experimental procedures of the performed animal experiments, and experimental procedure of the coupling of **8c** and **8d** (PDF)

Molecular formula string (XSLX)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

5-LOX, 5-lipoxygenase; AA, arachidonic acid; Ac, acetyl; ACN, acetonitrile; ATP, adenosine triphosphate; bs, broad singlet; bipy, 2,2′-bipyridine; Ccl2, chemokine (C−C motif) ligand 2; Col1a1, collagen I; COX, cyclooxygenase; CYP450, cytochrome P450; d, doublet; DCE, 1,2-dichloroethane; DCM, dichloromethane; DHETs, dihydroxyeicosatrienoic acids; DIAD, diisopropyl azodicarboxylate; DIPA, diisopropylamine; DIPEA, *N*,*N*-diisopropylethylamine; 4-DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; DML, multitarget ligand; DMSO, dimethyl sulfoxide; DPBS, Dulbecco's phosphate-buffered saline; dr, diastereomeric ratio; EETs, epoxyeicosatrienoic acids; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; EDTA, ethylenediaminetetraacetic acid; EtOH, ethanol; ESI, electrospray ionization; equiv, equivalent; FN1, fibronectin-1; FLAP, 5-lipoxygenase activating protein; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectrometry; IC_{50}, half maximal inhibitory concentration; J, coupling constant; LPS, lipopolysaccharide; LTs, leukotrienes; LTE_4, leukotriene E_4; LTB_4, leukotriene B_4; LXs, lipoxins; MeOH, methanol; μw, microwave; MS, mass spectrometry; m, multiplet; *n*BuLi, *n*-butyllithium; NMR, nuclear magnetic resonance; NSAIDs, non-steroidal anti-inflammatory drugs; PG, glucose; PGs, prostaglandins; PHOME, 3-phenyl-cyano(6-methoxy-2-naphthalenyl)methyl ester-2-oxiraneacetic acid; PMNL, polymorphonuclear neutrophils; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; q, quartet; RLM, rat liver microsomes; s, singlet; TX, thromboxane; SAR, structure−activity relationship; sEH, soluble epoxide hydrolase; t, triplet; THF, tetrahydrofuran; TLC, thin-layer chromatography; Tween80, polysorbate 80; UPLC, ultra-performance liquid chromatography; UUO, unilateral ureteral obstruction

■ REFERENCES

(1) Chen, L.; Deng, H.; Cui, H.; Fang, J.; Zuo, Z.; Deng, J.; Li, Y.; Wang, X.; Zhao, L. Inflammatory Responses and Inflammation-Associated Diseases in Organs. *Oncotarget* **2018**, *9*, 7204−7218.
(2) Rådmark, O.; Werz, O.; Steinhilber, D.; Samuelsson, B. 5-Lipoxygenase, a Key Enzyme for Leukotriene Biosynthesis in Health and Disease. *Biochim. Biophys. Acta* **2015**, *1851*, 331−339.
(3) Haeggström, J. Z. Leukotriene Biosynthetic Enzymes as Therapeutic Targets. *J. Clin. Invest.* **2018**, *128*, 2680−2690.
(4) Hiesinger, K.; Wagner, K. M.; Hammock, B. D.; Proschak, E.; Hwang, S. H. Development of Multitarget Agents Possessing Soluble

Epoxide Hydrolase Inhibitory Activity. *Prostaglandins Other Lipid Mediators* **2019**, *140*, 31−39.
(5) Hoxha, M.; Zappacosta, B. CYP-Derived Eicosanoids: Implications for Rheumatoid Arthritis. *Prostaglandins Other Lipid Mediators* **2020**, *146*, 106405.
(6) Wagner, K. M.; McReynolds, C. B.; Schmidt, W. K.; Hammock, B. D. Soluble Epoxide Hydrolase as a Therapeutic Target for Pain, Inflammatory and Neurodegenerative Diseases. *Pharmacol. Ther.* **2017**, *180*, 62−76.
(7) McGettigan, P.; Henry, D. Use of Non-Steroidal Anti-Inflammatory Drugs That Elevate Cardiovascular Risk: An Examination of Sales and Essential Medicines Lists in Low-, Middle-, and High-Income Countries. *PLoS Med.* **2013**, *10*, No. e1001388.
(8) Becker, J. C.; Domschke, W.; Pohle, T. Current Approaches to Prevent NSAID-Induced Gastropathy–COX Selectivity and Beyond. *Br. J. Clin. Pharmacol.* **2004**, *58*, 587−600.
(9) Szczeklik, A. The Cyclooxygenase Theory of Aspirin-Induced Asthma. *Eur. Respir. J.* **1990**, *3*, 588−593.
(10) Jung, O.; Jansen, F.; Mieth, A.; Barbosa-Sicard, E.; Pliquett, R. U.; Babelova, A.; Morisseau, C.; Hwang, S. H.; Tsai, C.; Hammock, B. D.; Schaefer, L.; Geisslinger, G.; Amann, K.; Brandes, R. P. Inhibition of the Soluble Epoxide Hydrolase Promotes Albuminuria in Mice with Progressive Renal Disease. *PLoS One* **2010**, *5*, No. e11979.
(11) Sala, A.; Proschak, E.; Steinhilber, D.; Rovati, G. E. Two-Pronged Approach to Anti-Inflammatory Therapy through the Modulation of the Arachidonic Acid Cascade. *Biochem. Pharmacol.* **2018**, *158*, 161−173.
(12) Liu, J.-Y.; Yang, J.; Inceoglu, B.; Qiu, H.; Ulu, A.; Hwang, S.-H.; Chiamvimonvat, N.; Hammock, B. D. Inhibition of Soluble Epoxide Hydrolase Enhances the Anti-Inflammatory Effects of Aspirin and 5-Lipoxygenase Activation Protein Inhibitor in a Murine Model. *Biochem. Pharmacol.* **2010**, *79*, 880−887.
(13) Temml, V.; Garscha, U.; Romp, E.; Schubert, G.; Gerstmeier, J.; Kutil, Z.; Matuszczak, B.; Waltenberger, B.; Stuppner, H.; Werz, O.; Schuster, D. Discovery of the First Dual Inhibitor of the 5-Lipoxygenase-Activating Protein and Soluble Epoxide Hydrolase Using Pharmacophore-Based Virtual Screening. *Sci. Rep.* **2017**, *7*, 42751.
(14) Garscha, U.; Romp, E.; Pace, S.; Rossi, A.; Temml, V.; Schuster, D.; König, S.; Gerstmeier, J.; Liening, S.; Werner, M.; Atze, H.; Wittmann, S.; Weinigel, C.; Rummler, S.; Scriba, G. K.; Sautebin, L.; Werz, O. Pharmacological Profile and Efficiency in Vivo of Diflapolin, the First Dual Inhibitor of 5-Lipoxygenase-Activating Protein and Soluble Epoxide Hydrolase. *Sci. Rep.* **2017**, *7*, 9398.
(15) Meirer, K.; Glatzel, D.; Kretschmer, S.; Wittmann, S. K.; Hartmann, M.; Blöcher, R.; Angioni, C.; Geisslinger, G.; Steinhilber, D.; Hofmann, B.; Fürst, R.; Proschak, E. Design, Synthesis and Cellular Characterization of a Dual Inhibitor of 5-Lipoxygenase and Soluble Epoxide Hydrolase. *Molecules* **2016**, *22*, 45.
(16) Hiesinger, K.; Schott, A.; Kramer, J. S.; Blöcher, R.; Witt, F.; Wittmann, S. K.; Steinhilber, D.; Pogoryelov, D.; Gerstmeier, J.; Werz, O.; Proschak, E. Design of Dual Inhibitors of Soluble Epoxide Hydrolase and LTA4 Hydrolase. *ACS Med. Chem. Lett.* **2019**, 298.
(17) Hwang, S. H.; Wagner, K. M.; Morisseau, C.; Liu, J.-Y.; Dong, H.; Wecksler, A. T.; Hammock, B. D. Synthesis and Structure-Activity Relationship Studies of Urea-Containing Pyrazoles as Dual Inhibitors of Cyclooxygenase-2 and Soluble Epoxide Hydrolase. *J. Med. Chem.* **2011**, *54*, 3037−3050.
(18) Zhang, G.; Panigrahy, D.; Hwang, S. H.; Yang, J.; Mahakian, L. M.; Wettersten, H. I.; Liu, J.-Y.; Wang, Y.; Ingham, E. S.; Tam, S.; Kieran, M. W.; Weiss, R. H.; Ferrara, K. W.; Hammock, B. D. Dual Inhibition of Cyclooxygenase-2 and Soluble Epoxide Hydrolase Synergistically Suppresses Primary Tumor Growth and Metastasis. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 11127−11132.
(19) Gartung, A.; Yang, J.; Sukhatme, V. P.; Bielenberg, D. R.; Fernandes, D.; Chang, J.; Schmidt, B. A.; Hwang, S. H.; Zurakowski, D.; Huang, S.; Kieran, M. W.; Hammock, B. D.; Panigrahy, D. Suppression of Chemotherapy-Induced Cytokine/Lipid Mediator Surge and Ovarian Cancer by a Dual COX-2/SEH Inhibitor. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 1698−1703.
(20) Zhang, C.-Y.; Duan, J.-X.; Yang, H.-H.; Sun, C.-C.; Zhong, W.-J.; Tao, J.-H.; Guan, X.-X.; Jiang, H.-L.; Hammock, B. D.; Hwang, S. H.; Zhou, Y.; Guan, C.-X. COX-2/SEH Dual Inhibitor PTUPB Alleviates Bleomycin-Induced Pulmonary Fibrosis in Mice via Inhibiting Senescence. *FEBS J.* **2019**, 1666.
(21) Dileepan, M.; Rastle-Simpson, S.; Greenberg, Y.; Wijesinghe, D. S.; Kumar, N. G.; Yang, J.; Hwang, S. H.; Hammock, B. D.; Sriramarao, P.; Rao, S. P. Effect Of Dual SEH/COX-2 Inhibition on Allergen-Induced Airway Inflammation. *Front. Pharmacol.* **2019**, *10*, 1118.
(22) Brooks, C. D.; Stewart, A. O.; Basha, A.; Bhatia, P.; Ratajczyk, J. D.; Martin, J. G.; Craig, R. A.; Kolasa, T.; Bouska, J. B.; Lanni, C. (R)-(+)-N-[3-[5-[(4-Fluorophenyl)Methyl]-2-Thienyl]-1-Methyl- 2-Propynyl]-N-Hydroxyurea (ABT-761), a Second-Generation 5-Lipoxygenase Inhibitor. *J. Med. Chem.* **1995**, *38*, 4768−4775.
(23) Podolin, P. L.; Bolognese, B. J.; Foley, J. F.; Long, E.; Peck, B.; Umbrecht, S.; Zhang, X.; Zhu, P.; Schwartz, B.; Xie, W.; Quinn, C.; Qi, H.; Sweitzer, S.; Chen, S.; Galop, M.; Ding, Y.; Belyanskaya, S. L.; Israel, D. I.; Morgan, B. A.; Behm, D. J.; Marino, J. P.; Kurali, E.; Barnette, M. S.; Mayer, R. J.; Booth-Genthe, C. L.; Callahan, J. F. In Vitro and in Vivo Characterization of a Novel Soluble Epoxide Hydrolase Inhibitor. *Prostaglandins Other Lipid Mediators* **2013**, *104-105*, 25−31.
(24) Klapars, A.; Buchwald, S. L. Copper-Catalyzed Halogen Exchange in Aryl Halides: An Aromatic Finkelstein Reaction. *J. Am. Chem. Soc.* **2002**, *124*, 14844−14845.
(25) van der Bent, A.; Blommaert, A. G.; Melman, C. T.; IJzerman, A. P.; van Wijngaarden, I.; Soudijn, W. Hybrid Cholecystokinin-A Antagonists Based on Molecular Modeling of Lorglumide and L-364,718. *J. Med. Chem.* **1992**, *35*, 1042−1049.
(26) Gribble, G. W. Sodium Borohydride and Carboxylic Acids: A Novel Reagent Combination. In *Reductions in Organic Synthesis*; ACS Symposium Series; American Chemical Society, 1996; Vol. *641*, pp. 167−200.
(27) Zhi, W.; Li, J.; Zou, D.; Wu, Y.; Wu, Y. Palladium-Catalyzed Diastereoselective Synthesis of *β*,*β*-Diarylpropionic Acid Derivatives and Its Application to the Total Synthesis of (R)-Tolterodine and the Enantiomer of a Key Intermediate for MK-8718. *Tetrahedron Lett.* **2018**, *59*, 537−540.
(28) Wolf, N. M.; Morisseau, C.; Jones, P. D.; Hock, B.; Hammock, B. D. Development of a High-Throughput Screen for Soluble Epoxide Hydrolase Inhibition. *Anal. Biochem.* **2006**, *355*, 71−80.
(29) Kretschmer, S. B. M.; Woltersdorf, S.; Vogt, D.; Lillich, F. F.; Rühl, M.; Karas, M.; Maucher, I. V.; Roos, J.; Häfner, A.-K.; Kaiser, A.; Wurglics, M.; Schubert-Zsilavecz, M.; Angioni, C.; Geisslinger, G.; Stark, H.; Steinhilber, D.; Hofmann, B. Characterization of the Molecular Mechanism of 5-Lipoxygenase Inhibition by 2-Aminothiazoles. *Biochem. Pharmacol.* **2017**, *123*, 52−62.
(30) Xie, Y.; Liu, Y.; Gong, G.; Smith, D. H.; Yan, F.; Rinderspacher, A.; Feng, Y.; Zhu, Z.; Li, X.; Deng, S.-X.; Branden, L.; Vidović, D.; Chung, C.; Schürer, S.; Morisseau, C.; Hammock, B. D.; Landry, D. W. Discovery of Potent Non-Urea Inhibitors of Soluble Epoxide Hydrolase. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2354−2359.
(31) Blöcher, R.; Lamers, C.; Wittmann, S. K.; Merk, D.; Hartmann, M.; Weizel, L.; Diehl, O.; Brüggerhoff, A.; Boß, M.; Kaiser, A.; Schader, T.; Göbel, T.; Grundmann, M.; Angioni, C.; Heering, J.; Geisslinger, G.; Wurglics, M.; Kostenis, E.; Brüne, B.; Steinhilber, D.; Schubert-Zsilavecz, M.; Kahnt, A. S.; Proschak, E. N-Benzylbenzamides: A Novel Merged Scaffold for Orally Available Dual Soluble Epoxide Hydrolase/Peroxisome Proliferator-Activated Receptor *γ* Modulators. *J. Med. Chem.* **2016**, *59*, 61−81.
(32) Thalji, R. K.; McAtee, J. J.; Belyanskaya, S.; Brandt, M.; Brown, G. D.; Costell, M. H.; Ding, Y.; Dodson, J. W.; Eisennagel, S. H.; Fries, R. E.; Gross, J. W.; Harpel, M. R.; Holt, D. A.; Israel, D. I.; Jolivette, L. J.; Krosky, D.; Li, H.; Lu, Q.; Mandichak, T.; Roethke, T.; Schnackenberg, C. G.; Schwartz, B.; Shewchuk, L. M.; Xie, W.; Behm, D. J.; Douglas, S. A.; Shaw, A. L.; Marino, J. P. Discovery of 1-(1,3,5-

https://dx.doi.org/10.1021/acs.jmedchem.0c00561
J. Med. Chem. XXXX, XXX, XXX−XXX

Triazin-2-Yl)Piperidine-4-Carboxamides as Inhibitors of Soluble Epoxide Hydrolase. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3584−3588.
(33) Eldrup, A. B.; Soleymanzadeh, F.; Taylor, S. J.; Muegge, I.; Farrow, N. A.; Joseph, D.; McKellop, K.; Man, C. C.; Kukulka, A.; De Lombaert, S. Structure-Based Optimization of Arylamides as Inhibitors of Soluble Epoxide Hydrolase. *J. Med. Chem.* **2009**, *52*, 5880−5895.
(34) Gilbert, N. C.; Gerstmeier, J.; Schexnaydre, E. E.; Börner, F.; Garscha, U.; Neau, D. B.; Werz, O.; Newcomer, M. E. Structural and Mechanistic Insights into 5-Lipoxygenase Inhibition by Natural Products. *Nat. Chem. Biol.* **2020**, *16*, 783−790.
(35) Duffield, J. S. Macrophages and Immunologic Inflammation of the Kidney. *Semin. Nephrol.* **2010**, *30*, 234−254.
(36) Stewart, A. O.; Brooks, D. W. N,O-Bis(Phenoxycarbonyl)-Hydroxylamine: A New Reagent for the Direct Synthesis of Substituted N-Hydroxyureas. *J. Org. Chem.* **1992**, *57*, 5020−5023.
(37) Hahn, S.; Achenbach, J.; Buscató, E.; Klingler, F.-M.; Schroeder, M.; Meirer, K.; Hieke, M.; Heering, J.; Barbosa-Sicard, E.; Loehr, F.; Fleming, I.; Doetsch, V.; Schubert-Zsilavecz, M.; Steinhilber, D.; Proschak, E. Complementary Screening Techniques Yielded Fragments That Inhibit the Phosphatase Activity of Soluble Epoxide Hydrolase. *ChemMedChem* **2011**, *6*, 2146−2149.
(38) Lukin, A.; Kramer, J.; Hartmann, M.; Weizel, L.; Hernandez-Olmos, V.; Falahati, K.; Burghardt, I.; Kalinchenkova, N.; Bagnyukova, D.; Zhurilo, N.; Rautio, J.; Forsberg, M.; Ihalainen, J.; Auriola, S.; Leppänen, J.; Konstantinov, I.; Pogoryelov, D.; Proschak, E.; Dar'in, D.; Krasavin, M. Discovery of Polar Spirocyclic Orally Bioavailable Urea Inhibitors of Soluble Epoxide Hydrolase. *Bioorg. Chem.* **2018**, *80*, 655−667.
(39) Hiesinger, K.; Kramer, J. S.; Achenbach, J.; Moser, D.; Weber, J.; Wittmann, S. K.; Morisseau, C.; Angioni, C.; Geisslinger, G.; Kahnt, A. S.; Kaiser, A.; Proschak, A.; Steinhilber, D.; Pogoryelov, D.; Wagner, K.; Hammock, B. D.; Proschak, E. Computer-Aided Selective Optimization of Side Activities of Talinolol. *ACS Med. Chem. Lett.* **2019**, *10*, 899−903.
(40) Brungs, M.; Rådmark, O.; Samuelsson, B.; Steinhilber, D. Sequential Induction of 5-Lipoxygenase Gene Expression and Activity in Mono Mac 6 Cells by Transforming Growth Factor Beta and 1,25-Dihydroxyvitamin D3. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 107−111.
(41) Steinhilber, D.; Herrmann, T.; Roth, H. J. Separation of Lipoxins and Leukotrienes from Human Granulocytes by High-Performance Liquid Chromatography with a Radial-Pak Cartridge after Extraction with an Octadecyl Reversed-Phase Column. *J. Chromatogr. B: Biomed. Sci. Appl.* **1989**, *493*, 361−366.
(42) Werz, O.; Bürkert, E.; Samuelsson, B.; Rådmark, O.; Steinhilber, D. Activation of 5-Lipoxygenase by Cell Stress Is Calcium Independent in Human Polymorphonuclear Leukocytes. *Blood* **2002**, *99*, 1044−1052.
(43) Xing, L.; McDonald, J. J.; Kolodziej, S. A.; Kurumbail, R. G.; Williams, J. M.; Warren, C. J.; O'Neal, J. M.; Skepner, J. E.; Roberds, S. L. Discovery of Potent Inhibitors of Soluble Epoxide Hydrolase by Combinatorial Library Design and Structure-Based Virtual Screening. *J. Med. Chem.* **2011**, *54*, 1211−1222.
(44) Kabsch, W. Xds. *Acta Cryst.* **2010**, *66*, 125−132.
(45) Adams, P. D.; Afonine, P. V.; Bunkóczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L.-W.; Kapral, G. J.; Grosse-Kunstleve, R. W. PHENIX: A Comprehensive Python-Based System for Macromolecular Structure Solution. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2010**, *66*, 213−221.
(46) Öster, L.; Tapani, S.; Xue, Y.; Käck, H. Successful Generation of Structural Information for Fragment-Based Drug Discovery. *Drug Discovery Today* **2015**, *20*, 1104−1111.
(47) Emsley, P.; Cowtan, K. Coot: Model-Building Tools for Molecular Graphics. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2004**, *60*, 2126−2132.
(48) Moriarty, N. W.; Grosse-Kunstleve, R. W.; Adams, P. D. Electronic Ligand Builder and Optimization Workbench (ELBOW): A Tool for Ligand Coordinate and Restraint Generation. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2009**, *65*, 1074−1080.
(49) Schierle, S.; Flauaus, C.; Heitel, P.; Willems, S.; Schmidt, J.; Kaiser, A.; Weizel, L.; Goebel, T.; Kahnt, A. S.; Geisslinger, G.; Steinhilber, D.; Wurglics, M.; Rovati, G. E.; Schmidtko, A.; Proschak, E.; Merk, D. Boosting Anti-Inflammatory Potency of Zafirlukast by Designed Polypharmacology. *J. Med. Chem.* **2018**, *61*, 5758−5764.
(50) Schwalm, S.; Beyer, S.; Frey, H.; Haceni, R.; Grammatikos, G.; Thomas, D.; Geisslinger, G.; Schaefer, L.; Huwiler, A.; Pfeilschifter, J. Sphingosine Kinase-2 Deficiency Ameliorates Kidney Fibrosis by Up-Regulating Smad7 in a Mouse Model of Unilateral Ureteral Obstruction. *Am. J. Pathol.* **2017**, *187*, 2413−2429.

https://dx.doi.org/10.1021/acs.jmedchem.0c00561
J. Med. Chem. XXXX, XXX, XXX−XXX