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# Active site mapping of trypsin, thrombin and matriptase-2 by sulfamoyl benzamidines

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ABSTRACT

The benzamidine moiety, a well-known arginine mimetic, has been introduced in a variety of ligands, including peptidomimetic inhibitors of trypsin-like serine proteases. According to their primary substrate specificity, the benzamidine residue interacts with the negatively charged aspartate at the bottom of the S1 pocket of such enzymes. Six series of benzamidine derivatives (**1**–**73**) were synthesized and evaluated as inhibitors of two prototype serine proteases, that is, bovine trypsin and human thrombin. As a further target, human matriptase-2, a recently discovered type II transmembrane serine protease, was investigated. Matriptase-2 represents an important regulatory protease in iron homeostasis by down-regulation of the hepcidin expression. Compounds **1**–**73** were designed to contain a fixed sulfamoyl benzamidine moiety as arginine mimetic and a linker-connected additional substructure, such as a *tert*-butyl ester, carboxylate or second benzamidine functionality. A systematic mapping approach was performed with these inhibitors to scan the active site of the three target proteases. In particular, bisbenzamidines, able to interact with both the S1 and S3/S4 binding sites, showed notable affinity. In branched bisbenzamidines **66**–**73** containing a third hydrophobic residue, opposite effects of the stereochemistry on trypsin and thrombin inhibition were observed.



## 1. Introduction

The benzamidine moiety is an important pharmacophore in medicinal chemistry prone to interact with protein regions bearing anionic and hydrogen bond acceptor groups. To improve their oral bioavailability, amidoximes have been successfully introduced as prodrugs which are metabolized to the active benzamidines in vivo by cytochrome P450 or other NAD(P)H-dependent oxidoreductases.[1]

Several synthetic routes to benzamidines have been reported, such as the conversion of aromatic imidates (Pinner reaction)[2–6] or thioimidates[5,7–11] by amination. The reaction of benzonitriles with aluminum amides, prepared from trimethylaluminum,[12,13] or lithium bis(trimethylsilyl)amide also proved to furnish benzamidines.[14–16] Furthermore, they are accessible via catalytic reduction of amidoximes, obtained from nitriles, by taking advantage of potassium or ammonium formate as hydrogen source.[13,17] This method was been applied to the synthesis on solid-phase support by reducing polymer-bound amidoximes using $SnCl_2 \times 2H_2O$.[18] Benzamidines can also be obtained by catalytic hydrogenation of *O*-acetylamidoximes,[19–23] a protocol that was utilized in the course of this study.

The bisbenzamidine scaffold exhibits a broad spectrum of antiprotozoal activity, mainly directed against the parasites *Plasmodium falciparum*, *Trypanosoma brucei*, and *Leishmania donovani*, which cause considerable morbidity and mortality,[24,25] as well as antifungal activity beneficial for the treatment of AIDS-related *Pneumocystis carinii* pneumonia.[25,26]

Benzamidines have been shown to act as excellent arginine side chain mimetics[27] to address targets where arginine is involved as a part of their natural ligand binding sequence. For example, benzamidines based upon the Arg-Gly-Asp sequence of the α-chain of fibrinogen are antagonists of the platelet membrane glycoproteins IIb/IIIa, that is, the fibrinogen receptors.[8,28] Benzamidine containing imidazolyl derivatives act as antagonists of the presynaptic histamine $H_3$ receptor.[13]

Because of the arginine mimicking properties, benzamidine substructures have been extensively utilized in the rational design of inhibitors for trypsin-like serine proteases which are characterized by their primary substrate specificity for arginine in P1 position. Examples include inhibitors for the target enzymes trypsin,[9] thrombin,[4,5] factor Xa,[6,10,11,16,21,29,30] urokinase,[22] plasmin,[31] and matriptase-1.[23] Among them, the thrombin inhibitors melagatran and dabigatran were introduced into the market, in form of their

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double prodrugs.[32,33] A binding mode for benzamidine inhibitors and trypsin-like serine proteases has been experimentally confirmed and is generally accepted, inasmuch as the benzamidine group is deeply buried in the S1 specificity pocket where a negatively charged conserved Asp189 at the bottom is engaged in a salt bridge with the positively charged benzamidine functionality.[34,35] The architecture of the S1 pocket of trypsin and thrombin resembles that of matriptase-2, a recently discovered serine protease. As trypsin and thrombin, matriptase-2 has a primary substrate specificity for basic amino acids in P1 position.[36–39] A homology model of matriptase-2 was generated showing that binding of arginine to the S1 site is likewise stabilized by a salt bridge to the specificity determining residue Asp756 (chymotrypsinogen numbering 189).[39]

Matriptase-2 belongs to the type II transmembrane serine proteases (TTSPs). One member of this family of cell surface proteases, matriptase-1, possesses an extracellular matrix degrading activity and represents a potential therapeutic target for cancer therapy.[23,40] The closely related enzyme matriptase-2, encoded by the gene *TMPRSS6*, is predominantly expressed in the liver and consists of a short N-terminal cytoplasmic tail, a single transmembrane domain, and a composite ectodomain with a C-terminal serine protease domain.[36,41] In vitro, matriptase-2 can degrade extracellular matrix components such as fibronectin, fibrinogen and type I collagen, but with lower efficiency than matriptase-1.[36] However, enhanced expression of matriptase-2 significantly reduced the invasive breast and prostate cancer growth, and decreased levels of matriptase-2 correlate with poor patient outcome, suggesting that matriptase-2 participates in regulating cellular migration.[42] The recently discovered role of matriptase-2 in iron homoeostasis has attracted much attention. Mutations in *TMPRSS6* lead to a loss of matriptase-2 proteolytic activity and are associated with iron-refractory iron-deficiency anemia (IRIDA) due to elevated levels of the liver hormone hepcidin.[43] Matriptase-2 inhibits hepcidin synthesis at the transcriptional level through proteolytic cleavage of the co-receptor hemojuvelin suppressing SMAD signaling which results in increased plasma iron levels.[44] A cell membrane localization of an inactive zymogen has been demonstrated using HEK-293 cells, stably transfected with cDNA encoding human matriptase-2.[38] Matriptase-2 undergoes shedding via a trans mechanism and accumulates in the conditioned medium as an activated form containing the catalytic domain. The conditioned medium was used as a source for matriptase-2 activity to determine $K_i$ values of the first matriptase-2 inhibitors.[39] Matriptase-2 inhibitors could be beneficial as pharmacological tools to further investigate its exact role in regulating iron homeostasis and might also be useful for therapeutic intervention of frequent iron disorders such as systemic iron overload (hemochromatosis) or iron loading anemias where the level of hepcidin is inappropriately low.

Herein, we present a combinatorial approach to map the active site of trypsin-like serine proteases with polyfunctionalized benzamidines. The compounds were designed to contain a fixed sulfamoyl benzamidine moiety for accommodation in the S1 pocket and several linker-connected functionalities for additional interactions with the S3/S4 binding region. We have prepared six series of benzamidine derivatives and evaluated these compounds towards three selected serine proteases, including matriptase-2, in fluorometric enzyme assays.

## 2. Results and discussion

Our initial results towards the synthesis of benzamidines indicated the conversion of nitriles to be the most practical synthetic route.[19] Thus, we considered 3- and 4-cyanobenzenesulfonyl chloride to be attractive building blocks for the formation of benzamidines as well as the introduction of the amide bioisosteric sulfonamide moiety.[45] Throughout the various series of products, the *meta*- or *para*-substitution pattern was incorporated into the benzamidine products as a first point of diversity.

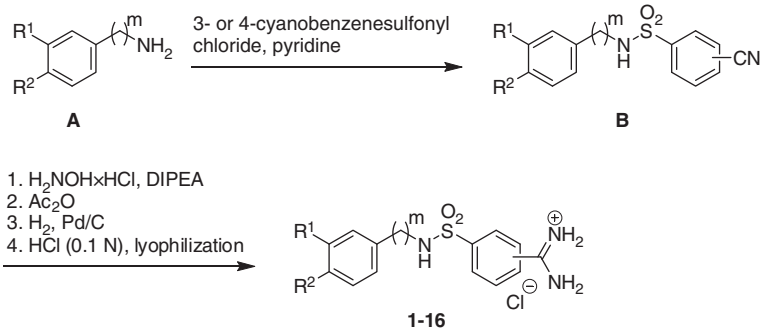
Sulfonamides resulting from aromatic amines and cyanobenzenesulfonyl chlorides were found to react with a second sulfonyl chloride in the presence of DIPEA to produce undesired bis-sulfonylated amines. Therefore, pyridine, as a weaker base, was used to synthesize sulfonamides **B** (Table 1). Compounds **B** were reacted with hydroxylamine, and the intermediate amidoximes were directly acetylated, followed by palladium-catalyzed hydrogenolysis. The process was optimized in terms of repressing the formation of 1,2,4-oxadiazole by-products.[46] Benzamidines **1**–**16** were efficiently precipitated either as acetate salts or as dipolar ions in excellent purity by addition of deep cooled, dry ethyl acetate to the oily residue which was obtained after filtration and evaporation of the solvent of the reduction step. Finally, lyophilization from aqueous HCl furnished the benzamidine hydrochlorides. With this powerful method of isolation in hand, we could use it for the other types of benzamidines (Tables 2–6) and adapt it to their physico-chemical properties. The majority of these products, that is, **5**–**16**, inhibited bovine trypsin with $K_i$ values between 16 and 50 μM. The binding affinities of **1**–**16** to thrombin were weak with a slight preference for the *meta*-substituted benzamidines. Matriptase-2 was not affected by these small benzamidines, except by compound **1** exhibiting a $K_i$ value of 88 μM.

To further map the active sites of the three proteases, we designed molecules which still contain the sulfamoyl benzamidine part connected via linkers of various lengths to a dimethoxybenzene moiety. As this hydrophobic substructure was maintained, the following series (Table 2) might allow to estimate the influence of the linker structure on the biological activity. Amine hydrochlorides **D** were obtained from Boc-protected glycine, β-alanine and γ-aminobutyric acid **C**, respectively, which were coupled with 3,4-dimethoxyaniline or the more flexible 3,4-dimethoxybenzylamine using the mixed anhydride method and deprotected with gaseous hydrogen chloride. Pyridine had to be replaced by DIPEA for the formation of the sulfonamides **E**, which were converted to benzamidines according to a slightly modified protocol. Thereby, the oily residues, obtained after hydrogenolysis, were dissolved in acetic acid and rapidly injected into deep cooled, dry ethyl acetate. Gaseous hydrogen chloride was passed through the resulting solution to precipitate the benzamidine hydrochlorides **17**–**28**. Initial attempts to start the sequence with the unprotected amino acid and the sulfonyl chloride, followed by mixed anhydride coupling with the dimethoxy-substituted amine led to an additional carbamoylation at the sulfonamide nitrogen.

The inhibitory activity of benzamidines **17**–**28** towards trypsin was similar compared to that of **1**–**16**. However, the insertion of a linker led to a stronger affection of thrombin, in particular in *meta*-substituted compounds (**12** and **16** vs **23**–**28**). All of these *meta* derivatives were more active than their *para*-substituted isomers **17**–**22**. The same trends were observed for matriptase-2, where only two *meta* compounds, **26** and **28** with the extended γ-amino butyric acid-based linker ($n = 3$), were inactive.

To verify the account of the hydrophobic residue at the site opposite to the benzamidine, an additional set of compounds was synthesized (Table 3). Glycine derivatives **29**–**32** contain various groups in place of the dimethoxyphenyl moiety of **23**, which exhibited the lowest $K_i$ value of the previous series. By using aqueous trifluoroacetic acid, the final compounds **29**–**32** were obtained as trifluoroacetates after lyophilization. The replacement of the 3,4-dimethoxyphenyl by the less electron-rich phenyl (in **29**) or the 2-naphthyl ring with an extended π-system (in **30**) or the nonaromatic cyclohexyl ring (in **31**) resulted in similar activity profiles. Thus, π–π interactions seem not to be essential for a

**Table 1**
Synthesis and biological evaluation of benzamidines **1**–**16**



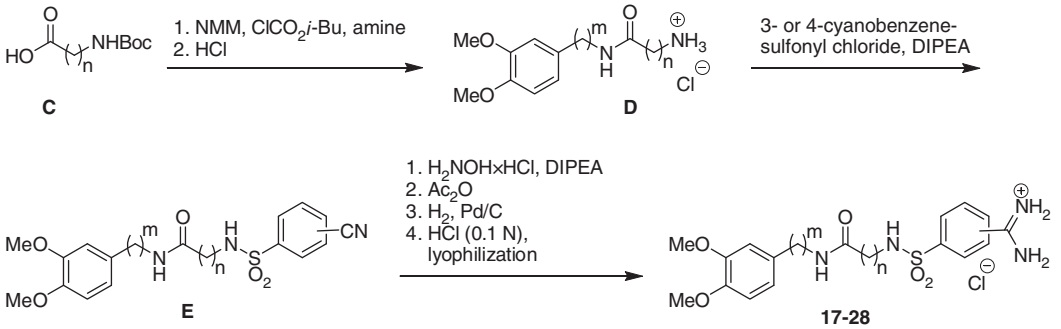

| Compd | $R^1$ | $R^2$ | *m* | Arom. subst. | Bovine trypsin $K_i$[a] (μM) | Human thrombin $K_i$[a] (μM) | Human matriptase-2 $K_i$[a] (μM) |
|---|---|---|---|---|---|---|---|
| **1** | H | H | 0 | *para* | n.i. | n.i. | 88 ± 4 |
| **2** | H | OMe | 0 | *para* | 135 ± 7 | n.i. | n.i. |
| **3** | OMe | H | 0 | *para* | n.i. | n.i. | n.i. |
| **4** | OMe | OMe | 0 | *para* | 150 ± 4 | n.i. | n.i. |
| **5** | H | H | 1 | *para* | 22 ± 2 | 230 ± 23 | n.i. |
| **6** | H | OMe | 1 | *para* | 16 ± 1 | n.i. | n.i. |
| **7** | OMe | H | 1 | *para* | 20 ± 1 | 223 ± 20 | n.i. |
| **8** | OMe | OMe | 1 | *para* | 20 ± 1 | n.i. | n.i. |
| **9** | H | H | 0 | *meta* | 49 ± 3 | 160 ± 18 | n.i. |
| **10** | H | OMe | 0 | *meta* | 44 ± 2 | 71 ± 3 | n.i. |
| **11** | OMe | H | 0 | *meta* | 45 ± 1 | 165 ± 3 | n.i. |
| **12** | OMe | OMe | 0 | *meta* | 50 ± 5 | 120 ± 4 | n.i. |
| **13** | H | H | 1 | *meta* | 36 ± 3 | 102 ± 8 | n.i. |
| **14** | H | OMe | 1 | *meta* | 44 ± 4 | 245 ± 20 | n.i. |
| **15** | OMe | H | 1 | *meta* | 55 ± 5 | 165 ± 11 | n.i. |
| **16** | OMe | OMe | 1 | *meta* | 34 ± 3 | n.i. | n.i. |

[a] n.i.: no inhibition relates to $IC_{50}$ values of >500 μM. $IC_{50}$ values were determined from duplicate measurements with five different inhibitor concentrations. The substrate concentration of 40 μM refers to 1.81 × $K_m$ (bovine trypsin), 1.01 × $K_m$ (human thrombin) and 1.24 × $K_m$ (matriptase-2). $K_i$ values were calculated using the equation $K_i = IC_{50}/(1+[S]/K_m)$.

proposed accommodation in the S3/S4 binding pocket of the proteases. The presence of the less bulky aliphatic isopropyl residue (in **32**) reduced the inhibitory activity against matriptase-2. A comparison of the amide linker-connected compounds **29** (Table 3) and **23** (Table 2) with their analogues lacking the amide group (**13** and **16**, Table 1) revealed an advantageous effect of the additional CONH unit present in **29** and **23**.

Next, we focused on a further modification of the linker structure connecting the *meta* benzamidine core with the hydrophobic moiety. As depicted in Table 4, Boc-glycine was reacted with

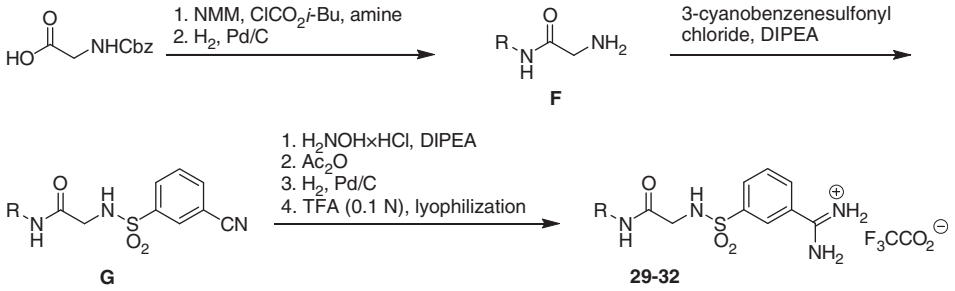
**Table 2**
Synthesis and biological evaluation of benzamidines **17**–**28** with dimethoxybenzene substructure




| Compd | *m* | *n* | Arom. Subst. | Bovine trypsin $K_i$[a] (μM) | Human thrombin $K_i$[a] (μM) | Human matriptase-2 $K_i$[a] (μM) |
|---|---|---|---|---|---|---|
| **17** | 0 | 1 | *para* | 11.4 ± 0.1 | 250 ± 26 | 115 ± 17 |
| **18** | 0 | 2 | *para* | 13.6 ± 0.6 | 187 ± 11 | n.i. |
| **19** | 1 | 1 | *para* | 52 ± 1 | 172 ± 8 | n.i. |
| **20** | 0 | 3 | *para* | 20.2 ± 0.6 | n.i. | n.i. |
| **21** | 1 | 2 | *para* | 26.9 ± 0.8 | 175 ± 8 | n.i. |
| **22** | 1 | 3 | *para* | 30 ± 2 | n.i. | n.i. |
| **23** | 0 | 1 | *meta* | 6.4 ± 0.4 | 15.6 ± 0.6 | 82 ± 10 |
| **24** | 0 | 2 | *meta* | 14.2 ± 0.2 | 32 ± 1 | 132 ± 16 |
| **25** | 1 | 1 | *meta* | 4.8 ± 0.3 | 12.3 ± 0.4 | 155 ± 24 |
| **26** | 0 | 3 | *meta* | 17 ± 1 | 83 ± 5 | n.i. |
| **27** | 1 | 2 | *meta* | 15.6 ± 0.9 | 28 ± 2 | 187 ± 30 |
| **28** | 1 | 3 | *meta* | 33 ± 2 | 60 ± 3 | n.i. |

[a] For assay details, see Table 1.

**Table 3**
Synthesis and biological evaluation of benzamidines **29–32** with modified hydrophobic substructures




| Compd | R | Bovine trypsin $K_i$[a] (μM) | Human thrombin $K_i$[a] (μM) | Human matriptase-2 $K_i$[a] (μM) |
|---|---|---|---|---|
| **29** | Ph | 10.9 ± 0.7 | 18 ± 1 | 65 ± 4 |
| **30** | 2-Naphthyl | 5.58 ± 0.09 | 4.0 ± 0.2 | 50 ± 4 |
| **31** | Cy | 12.6 ± 0.5 | 7.6 ± 0.8 | 64 ± 6 |
| **32** | *i*-Pr | 15 ± 1 | 13 ± 1 | 148 ± 6 |

[a] For assay details, see Table 1.

3-aminobenzonitrile and deprotected to the ammonium chloride **H** which was then converted with various sulfonyl chlorides to nitriles **I**. Following the protocol already applied for the previous four benzamidines, the products **33–35** were synthesized. Among them, **33** and **34** share the substitution pattern of benzamidines **23** (Table 2) and **30** (Table 3), respectively, but possess the inverted peptidomimetic chain. Actually, this modification diminished the affinity for trypsin and matriptase-2 (**33** vs **23**; **34** vs **30**). For the design of further compounds, we concluded to maintain the non-inverted linker structure and thus to continue using 3- and 4-cyanobenzenesulfonyl chloride as precursors for the P1 position.

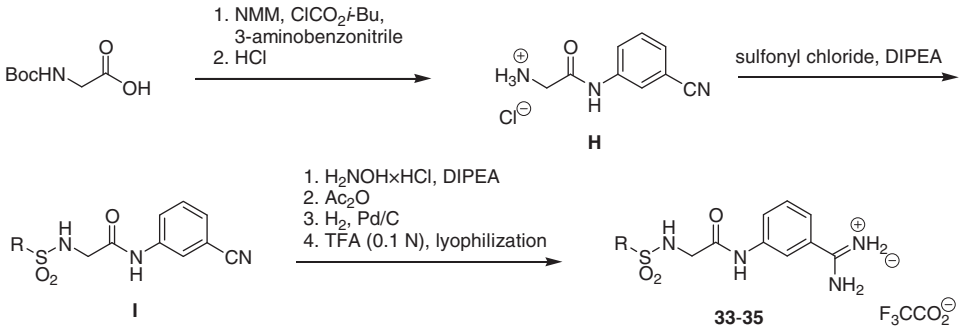
At this point of the study, we decided to systematically map the binding area of the S3/S4 pocket of the selected serine proteases by introducing a *tert*-butoxycarbonyl or a carboxy residue into the aromatic ring at the site opposite to the benzamidine. For this purpose, a complete combination was achieved with respect to three points of diversity, (i) the position of the *tert*-butoxycarbonyl and carboxy residues (3- or 4-position), (ii) the length of the linker ($n$ = 1–3), and (iii) the orientation of the amidine group (*para* or *meta*). The corresponding final products, *tert*-butyl benzoates **36–47** and benzoic acid derivatives **48–59** are displayed in Table 5. Coupling of the Cbz-protected amino acids **J** with *tert*-butyl 4-aminobenzoate or *tert*-butyl 3-aminobenzoate and deprotection afforded intermediates **K**. The required aniline derivatives were obtained from the respective nitrobenzoic acids upon esterification with oxalyl chloride and *tert*-butanol followed by reduction of the nitro group. The transformation into sulfamoyl benzamidines **36–47** was carried out via cyano-substituted sulfonamides **L**. The *tert*-butyl ester group was not affected under the conditions used for the preparation of **36–47**. The second group of test compounds, benzoic acid derivatives **48–59**, was prepared by trifluoroacetic acid-promoted cleavage of *tert*-butyl esters **36–47**. Overall, the route to **36–47** and **48–59** included eight or nine synthetic steps, respectively.

In general, trypsin was inhibited by these compounds to a similar extent as observed for the other benzamidines noted above. Rather minor differences in the trypsin-inhibiting activity of **36–59** were determined, irrespective of the presence of the ester or acid group, the linker structure and the positions of the aromatic substituents. Comparable trends in the structure-activity relationships of **36–59** were found both for thrombin and matriptase-2, as follows. The *tert*-butyl esters **36–47** showed an enhanced activity compared with the benzoic acid derivatives **48–59**. The affinities of the *meta*-substituted benzamidines exceeded those of the *para*-substituted ones. Consequently, the benzoic acid derivatives with a *para*-benzamidine substructure (**48–53**) are mainly inactive, whereas the *tert*-butyl benzoates with a *meta* benzamidine substructure (**42–47**) represent the most active inhibitors of this series. For matriptase-2 inhibition, a *tert*-butoxycarbonyl/carboxy substitution at position 4 was favored over position 3. Thus, the *meta* benzamidine **44** containing the β-alanine linker and the *tert*-butyl ester group at 4-position ($K_i$ = 7.6 μM) was identified as a substance inhibiting matriptase-2 in the lower micromolar range with some selectivity over thrombin. Noticeably, the best matriptase-2 inhibitor among the benzoic acid derivatives, **56** ($K_i$ = 52 μM), shares the substitution pattern of **44**. Within the group of 12 *meta* benzamidines, only **47** and **59** with an incorporated γ-amino butyric acid ($n$ = 3) did not inhibit matriptase-2. This finding reinforces the preference of matriptase-2 for shorter linker lengths (see Table 2, compounds **26** and **28**).

It could be concluded that the negatively charged carboxylic group did not contribute to attractive interactions between benzoic acid derivatives and the S3/S4 binding site of thrombin and matriptase-2. To investigate the outcome of positively charged substructures, we introduced a second benzamidine functionality, for which 4-cyanobenzylamine was used as precursor (Table 6). Besides glycine, β-alanine, and γ-aminobutyric acid ($R^1$ = $R^2$ = H, $n$ = 1–3), both enantiomers of phenylalanine and *O*-benzylserine ($n$ = 1) were selected to function as linker structures. Ammonium salts **N** were obtained via mixed anhydride method from Boc-protected amino acids **C** or **M**, respectively, and 4-cyanobenzylamine, followed by deprotection. Dinitriles **O** were obtained from **N** and 3- or 4-cyanobenzenesulfonyl chloride and were subsequently transformed to bisbenzamidines **60–73** using the standard protocol for the conversion of benzonitriles to benzamidines. The final products **60–73** were afforded after purification using a reversed phase HPLC.

The biological data of the unbranched bisbenzamidines, that is, **60–65**, were initially compared with the analogous dimethoxybenzyl derivatives (**17–28**, $m$ = 1; Table 2). Such a comparison allowed to assess the effect of an exchange of the dimethoxybenzene ring by the second benzamidine moiety for the interaction with the proposed S3/S4 binding pocket. In nearly all of the 18 cases, compounds **60–65** were more active than their dimethoxy counterparts at trypsin, thrombin, and matriptase-2, respectively. Examples for a pronounced improvement of the affinity include trypsin inhibition by **61** ($K_i$ = 0.53 vs 26.9 μM of **21**), thrombin inhibition by **65** ($K_i$ = 5.1 vs 60 μM of **28**), and matriptase 2 inhibition

**Table 4**
Synthesis and biological evaluation of benzamidines **33**–**35** with an inverted chain




H

I

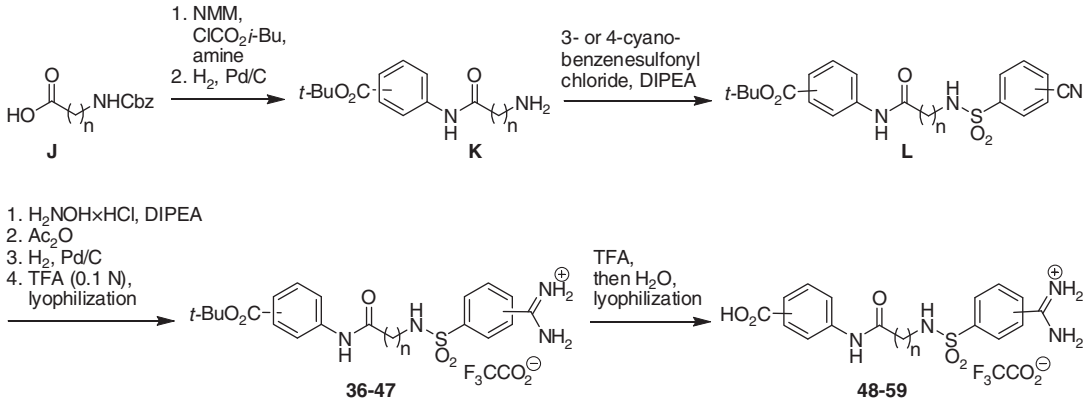
33-35

| Compd | R | Bovine trypsin $K_i$[a] (μM) | Human thrombin $K_i$[a] (μM) | Human matriptase-2 $K_i$[a] (μM) |
|---|---|---|---|---|
| **33** | 3,4-$(OMe)_2$Ph | 33 ± 1 | 10.6 ± 0.6 | 196 ± 7 |
| **34** | 2-Naphthyl | 29.4 ± 0.9 | 8.53 ± 0.41 | 163 ± 6 |
| **35** | 1-Naphthyl | 19.2 ± 0.9 | 5.82 ± 0.37 | 163 ± 2 |

[a] For assay details, see Table 1.

by **64** ($K_i$ = 8.9 vs 187 μM of **27**). Introduction of β-alanine or γ-aminobutyric acid ($n$ = 2 or 3) was found to be rather preferred for trypsin and thrombin than for matriptase-2 inhibition. Noteworthy, with respect to the three proteases and inhibitors **60**–**65**,

**Table 5**
Synthesis and biological evaluation of benzamidines **36**–**59** with *tert*-butyl benzoate and benzoic acid substructures




J

K

L

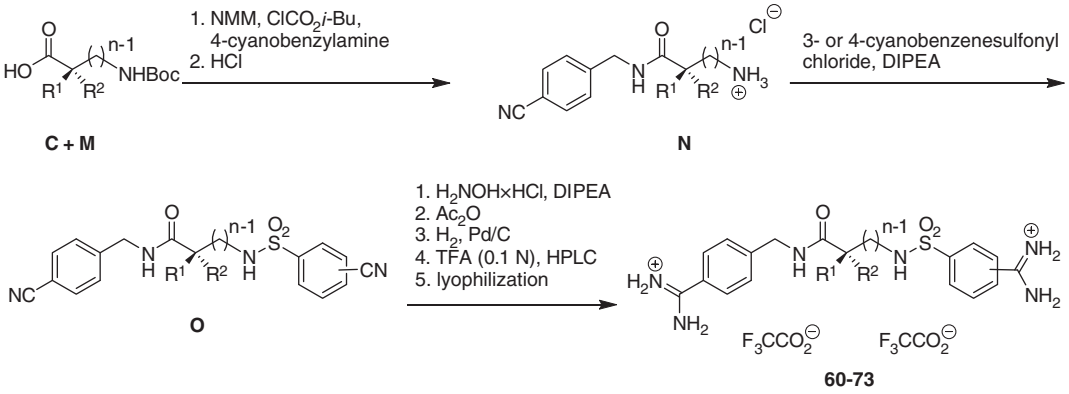
36-47

48-59

| Compd | $CO_2t$-Bu/$CO_2$H | $n$ | Arom. subst. | Bovine trypsin $K_i$[a] (μM) | Human thrombin $K_i$[a] (μM) | Human matriptase-2 $K_i$[a] (μM) |
|---|---|---|---|---|---|---|
| **36** | 4-$CO_2t$-Bu | 1 | *para* | 31 ± 2 | 85 ± 2 | 42 ± 2 |
| **37** | 3-$CO_2t$-Bu | 1 | *para* | 56 ± 2 | 64 ± 4 | n.i. |
| **38** | 4-$CO_2t$-Bu | 2 | *para* | 10.9 ± 0.6 | 174 ± 14 | n.i. |
| **39** | 3-$CO_2t$-Bu | 2 | *para* | 26.4 ± 0.7 | 100 ± 6 | n.i. |
| **40** | 4-$CO_2t$-Bu | 3 | *para* | 7.1 ± 0.1 | 103 ± 5 | 27 ± 1 |
| **41** | 3-$CO_2t$-Bu | 3 | *para* | 17 ± 2 | 115 ± 12 | n.i. |
| **42** | 4-$CO_2t$-Bu | 1 | *meta* | 7.9 ± 0.5 | 13.8 ± 0.8 | 137 ± 7 |
| **43** | 3-$CO_2t$-Bu | 1 | *meta* | 11.2 ± 0.3 | 7.4 ± 0.5 | 48 ± 4 |
| **44** | 4-$CO_2t$-Bu | 2 | *meta* | 16.7 ± 0.8 | 63 ± 4 | 7.6 ± 0.3 |
| **45** | 3-$CO_2t$-Bu | 2 | *meta* | 12.3 ± 0.3 | 13 ± 1 | 65 ± 4 |
| **46** | 4-$CO_2t$-Bu | 3 | *meta* | 12.2 ± 0.3 | 15 ± 1 | 70 ± 2 |
| **47** | 3-$CO_2t$-Bu | 3 | *meta* | 12.1 ± 0.9 | 30 ± 4 | n.i. |
| **48** | 4-$CO_2$H | 1 | *para* | 70 ± 1 | n.i. | n.i. |
| **49** | 3-$CO_2$H | 1 | *para* | 126 ± 3 | n.i. | n.i. |
| **50** | 4-$CO_2$H | 2 | *para* | 101 ± 5 | n.i. | n.i. |
| **51** | 3-$CO_2$H | 2 | *para* | 128 ± 13 | n.i. | n.i. |
| **52** | 4-$CO_2$H | 3 | *para* | 25.0 ± 0.6 | 66 ± 4 | n.i. |
| **53** | 3-$CO_2$H | 3 | *para* | 50 ± 2 | 201 ± 23 | n.i. |
| **54** | 4-$CO_2$H | 1 | *meta* | 21.9 ± 0.8 | 52 ± 10 | 139 ± 22 |
| **55** | 3-$CO_2$H | 1 | *meta* | 30.1 ± 0.9 | 37 ± 4 | 207 ± 25 |
| **56** | 4-$CO_2$H | 2 | *meta* | 26 ± 2 | 29 ± 2 | 52 ± 2 |
| **57** | 3-$CO_2$H | 2 | *meta* | 37 ± 1 | 88 ± 14 | 208 ± 8 |
| **58** | 4-$CO_2$H | 3 | *meta* | 28.2 ± 0.8 | 30 ± 2 | 217 ± 11 |
| **59** | 3-$CO_2$H | 3 | *meta* | 35 ± 1 | 75 ± 13 | n.i. |

[a] For assay details, see Table 1.

**Table 6**
Synthesis and biological evaluation of bisbenzamidines **60**–**73**




| Compd | $R^1$ | $R^2$ | *n* | *R*/*S* | Arom. subst. | Bovine trypsin $K_i$[a] (μM) | Human thrombin $K_i$[a] (μM) | Human matriptase-2 $K_i$[a] (μM) |
|---|---|---|---|---|---|---|---|---|
| **60** | H | H | 1 | — | *para* | 3.7 ± 0.3 | 125 ± 12 | 22 ± 1 |
| **61** | H | H | 2 | — | *para* | 0.53 ± 0.02 | 10.6 ± 0.3 | 19.0 ± 0.5 |
| **62** | H | H | 3 | — | *para* | 0.67 ± 0.04 | 7.4 ± 0.4 | 24 ± 1 |
| **63** | H | H | 1 | — | *meta* | 1.59 ± 0.08 | 17 ± 1 | 10.7 ± 0.6 |
| **64** | H | H | 2 | — | *meta* | 0.60 ± 0.20 | 8.2 ± 0.7 | 8.9 ± 0.8 |
| **65** | H | H | 3 | — | *meta* | 0.57 ± 0.04 | 5.1 ± 0.3 | 19 ± 2 |
| **66** | Bn | H | 1 | *S* | *para* | 0.42 ± 0.02 | 16.5 ± 0.8 | 11.8 ± 0.5 |
| **67** | H | Bn | 1 | *R* | *para* | 8.4 ± 0.2 | 6.0 ± 0.2 | 40 ± 1 |
| **68** | $CH_2OBn$ | H | 1 | *S* | *para* | 0.54 ± 0.05 | 32 ± 2 | 25.3 ± 0.7 |
| **69** | H | $CH_2OBn$ | 1 | *R* | *para* | 6.5 ± 0.2 | 0.93 ± 0.07 | 40 ± 1 |
| **70** | Bn | H | 1 | *S* | *meta* | 0.18 ± 0.01 | 10.7 ± 0.5 | 16.3 ± 0.7 |
| **71** | H | Bn | 1 | *R* | *meta* | 4.0 ± 0.2 | 2.4 ± 0.2 | 27 ± 1 |
| **72** | $CH_2OBn$ | H | 1 | *S* | *meta* | 0.26 ± 0.02 | 11.2 ± 0.9 | 24 ± 1 |
| **73** | H | $CH_2OBn$ | 1 | *R* | *meta* | 1.8 ± 0.1 | 0.53 ± 0.02 | 12.5 ± 0.4 |

[a] For assay details, see Table 1.

a positive impact for binding affinity caused by the *meta* substitution was not observed.

Based on the structures of the glycine-connected substances **60** (*para* substitution) and **63** (*meta* substitution) we designed branched bisbenzamidines, in which one backbone hydrogen atom was replaced by a benzyl or benzyloxymethylene residue ($n = 1$; R = Bn or $CH_2OBn$). The corresponding phenylalanine- and *O*-benzylserine-connected bisbenzamidines **66–73** bear a terminal aromatic core to potentially increase affinity to the proteases. Thus, the active site mapping was extended by hydrophobic interactions with putative binding areas, in addition to the polar interactions of the two benzamidine substructures. Depending on the stereochemical configuration of the branched bisbenzamidines, introduction of the hydrophobic side chain either improved the activity towards trypsin (**60** and **63** vs *S*-configured compounds **66**, **68** and **70**, **72**) or impaired it (**60** and **63** vs *R*-configured compounds **67**, **69** and **71**, **73**). In contrast, in the case of thrombin, *R*-configured derivatives **67**, **69**, **71**, **73** represented the eutomers and *S*-configured derivatives **66**, **68**, **70**, **72** the distomers. Each of the *meta*-substituted inhibitors was slightly more potent than the corresponding *para* compound on trypsin and thrombin. Next, the influence of the structure of the hydrophobic side chain was inspected. When comparing the corresponding eutomers on trypsin, phenylalanine and *O*-benzylserine derivatives were equipotent (**66**, **68**, **70**, **72**). With regard to thrombin inhibition, the *O*-benzylserine-connected eutomers **69** and **73** exhibited lower $K_i$ values (0.93 and 0.53 μM) probably owing to their larger size and higher flexibility. The phenylalanine-connected eutomers **67** and **71** were less potent (6.0 and 2.4 μM). Surprisingly, matriptase-2 catalysis was hardly affected by the introduction of the third branch into the bisbenzamidine framework (**66–73**). Significant differences between the corresponding *S*- and *R*-configured enantiomers were not detected. Moreover, *para*- or *meta*-substituted derivatives showed similar matriptase-2 inhibition and the structure of the hydrophobic side chain did not markedly influence the inhibitors' potency.

## 3. Conclusions

An active site mapping for three related serine proteases was performed. Throughout six series of sulfamoyl benzamidine inhibitors, an ascending level of complexity was achieved. We have systematically introduced several points of diversity, including the substitution pattern at the terminal aromatic cores, the nature of the connecting spacer and the attachment of a branch to the linker. A typical salt bridge between the amidino function and the carboxylate group of Asp189 situated in the S1 cavity is assumed. With a so anchored sulfamoyl benzamidine moiety at the S1 pocket, the impact of the substituent at the second aromatic ring could be elucidated. This part of the molecules is expected to scan the extended S3/S4 binding site of the three target enzymes. As an overall trend, the presence of a carboxyl group in the second aromatic ring was clearly unfavored. Both, a dimethoxy and a *tert*-butoxycarbonyl substitution led to an improved affinity towards trypsin, thrombin and matriptase-2. This finding can be attributed to the known hydrophobic character of the S3/S4 binding site of the proteases.

General observations indicate that trypsin-like enzymes favor ligands with large hydrophobic substituents in the S3/S4 pocket.[35,47] In trypsin, this region represents an aryl binding site. It is formed by Leu99 and Gly174 and the ethylene side chain of Gln175 and has Trp215 at its bottom. The N-terminal naphthylsulfonyl group of amidinophenylalanine amides is buried in this pocket.[32,34] In thrombin, the S3 pocket is rather flat and two aliphatic amino acids, Leu99 and Ile174, make up the S4 binding

site which is terminated by Trp215. Thus, the cyclohexyl moiety of melagatran orients to this region. Aromatic units also occupy the S4 pocket of thrombin,[34] for example the 2-pyridyl residue of dabigatran, as well as the arylsulfonyl group of NAPAP and argatroban.[32] Accordingly, the structure of the complex between the irreversible inhibitor D-Phe-Pro-Arg-chloromethylketone (PPACK) and thrombin shows the phenyl group in the S4 pocket.[48] In matriptase-2, the S3/S4 binding region is also hydrophobic in nature. Trp783 (chymotrypsinogen numbering 215) is still present, whereas the aliphatic-unpolar amino acids at positions 99 and 174 are exchanged for His665(99) and Tyr740(174). The S3/S4 pocket extents to Leu785(217) making this spot more hydrophobic compared to trypsin or thrombin which have Ser or Glu in the corresponding position. The cyclohexyl ring of one of the few known matriptase-2 inhibitors, that is, benzylsulfonyl-D-cyclohexylalanyl-proline-(4-amidinobenzyl)amide, might be positioned within the S3/S4 pocket for favorable interactions with Trp783(215) and Leu785(217), as has been proposed by docking studies.[39] The inhibitor exhibited an increased affinity towards a mutated variant of matriptase-2 in which Phe was introduced in place of His665(99), resulting in a pronounced hydrophobic character of the upper part of the S3/S4 pocket.[49]

Besides hydrophobic and π–π interactions, the S3/S4 pocket also enables polar and π-cation contacts with corresponding ligands.[6] In the complex of a bisbenzamidine inhibitor and trypsin, the one benzamidine group resides in the S1 pocket and the other one is anchored via a hydrogen-bonding network to the backbone oxygens of amino acids defining the S3/S4 binding site.[11] A similar binding mode was elucidated in a study on bisbenzamidines with a central isosorbide scaffold in the complexes with trypsin mutants.[35] In the case of thrombin, the S3 pocket is considered to provide ionic contacts to ligands, whereas the S4 pocket tends to accommodate lipophilic groups.[32] The model of matriptase-2 revealed a somewhat different feature in that the upper part of the S3/S4 pocket, formed by the backbone carbonyl oxygens of Glu662(96), Asp663(97) and Ser664(98), represents a negatively charged environment. The guanidino group of a second matriptase-2 inhibitor, that is, benzylsulfonyl-D-argininyl-proline-(4-amidinobenzyl)amide, was proposed to point to this region.[39]

The incorporation of a second benzamidine moiety was conducted to study the influence of these basic substructures (**60**–**73**). Indeed, in comparison with the acidic groups present in **48**–**59**, a clear overall improvement in affinity was achieved. These results reflect attractive interactions to the putative S3/S4 binding region of trypsin, thrombin and matriptase-2. Following our initial assumption on the binding mode of bisbenzamidines, the sulfamoyl benzamidine group is anchored in the S1 pocket and the aminomethyl benzamidine group resides in the S3/S4 cavity. However, a reversed orientation can be taken into consideration, because the preference for a *meta*-substitution at the sulfamoyl benzamidine portion is much less obvious for compounds **60**–**73**. Such a retro-binding mode was observed in trypsin complexes with two related bisbenzamidine inhibitors. In one case, the sulfamoyl benzamidine portion was inserted into the S1 pocket, whereas in the other one, the amidinophenylalanine side chain was bound in this pocket.[11]

Our study demonstrated unexpected opposite effects of the stereochemistry of branched bisbenzamidines on trypsin and thrombin inhibition. An incorporation of an *S*-configured, hydrophobic amino acid was advantageous for trypsin, whereas a contrary result was obtained for thrombin. The configuration of substrate-analogue inhibitors has a strong impact on their activity; but opposite effects on trypsin and thrombin have not been observed so far. For example, in 2-naphthylsulfonyl-(3-amidinophenyl)alanine-piperazides, the L-enantiomers were much more potent than the D-analogs against both, trypsin and thrombin.[5] A D-configured amino acid at P3 position is generally preferred in inhibitors of trypsin-like serine proteases containing an arginine mimetic as P1 amino acid and an L-amino acid at P2 position.[30–32,50] A branched substructure as present in **66**–**73** did not result in a benefit in terms of matriptase-2 inhibition, regardless of the stereochemistry. Those regions of the active site of matriptase-2 that can be reached by these hydrophobic branches do obviously not allow for attractive interactions. Thus, polar residues, in particular basic groups should be introduced into the bisbenzamidine scaffold for the design of improved inhibitors.

## 4. Experimental

### 4.1. General methods and materials

Melting points were determined on a Büchi B510 oil bath apparatus and are not corrected. Thin-layer chromatography was performed on Merck aluminum sheets, silica gel 60 $F_{254}$. Preparative column chromatography was performed on Acros silica gel (0.06–0.20 mm, 60 Å). $^{1}H$ and $^{13}C$ NMR spectra were acquired on a Bruker Avanced DRX 500 spectrometer operating at 500 MHz for $^{1}H$ and 125 MHz for $^{13}C$ at 25 °C. Chemical shifts $\delta$ are given in ppm referring to the signal center using the solvent peak for reference: DMSO-$d_6$ 2.49/39.7 ppm. The NMR signals were assigned by $^{1}H$, $^{13}C$ correlation spectra (HMQC, HMBC) using standard pulse sequences. Spin multiplicities are indicated by the following symbols: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), tt (triplet of triplet), q (quartet), sept (septet), and m (multiplet). The purity of all tested compounds was determined by HPLC–UV obtained on an LC–MS instrument (ABSCIEX API 2000 LC–MS/MS, HPLC Agilent 1100). A sample of 10 μL (0.5 mg, mL) was injected into a Phenomenex Luna C18 HPLC column (50 × 2.00 mm, particle size 3 μm) and was chromatographed at a flow rate of 250 μL/min using four different HPLC methods. Method a was as follows: The compound was dissolved in water. Mobile phase A, water with 2 mM ammonium acetate; B, methanol; gradient, 10% B in 20 min to 100% B, 10 min 100% B. Method b was as follows: The compound was dissolved in methanol. Mobile phase A, water with 10 mM ammonium formiate and 0.1% formic acid; B, methanol with 0.1% formic acid; gradient, 10% B in 20 min to 100% B, 10 min 100% B. Method c was as follows: The compound was dissolved in methanol. Mobile phase A, water with 10 mM ammonium formiate and 0.1% formic acid; B, methanol with 0.1% formic acid; gradient, 40% B in 20 min to 100% B, 10 min 100% B. Method d was as follows: The compound was dissolved in water. Mobile phase A, water with 10 mM ammonium formiate and 0.1% formic acid; B, methanol with 0.1% formic acid; gradient, 10% B in 20 min to 100% B, 10 min 100% B. UV absorption was detected from 200 to 950 nm using a diode array detector. Purity of the compounds was determined at 220–400 nm. Mass spectra were recorded on an API 2000 mass spectrometer (electron spray ion source, ABSCIEX, Darmstadt, Germany) coupled with an Agilent 1100 HPLC system. Elemental microanalyses were performed on a VarioEL apparatus. Enzymatic reactions were monitored in 96 well plates with flat bottom and lid (Sarstedt, Newton, USA) on a FLUOstar Optima fluorimeter (BMG LABTECH, Offenburg, Germany). The substrates Boc-Gln-Ala-Arg-NH-Mec (human matriptase-2, bovine trypsin) and Cbz-Gly-Gly-Arg-NH-Mec (human thrombin) were obtained from Bachem (Bubendorf, Switzerland). Bovine trypsin was purchased from Sigma and human thrombin from Calbiochem (Darmstadt, Germany). The conditioned medium of HEK-MT2 cells was used as source for matriptase-2 as described.[38,39] Commercial available 4-cyanobenzylamine (Sigma, Steinheim, Germany), 4-cyanobenzenesulfonyl chloride (Alfa Aesar, Karlsruhe, Germany) and 3-cyanobenzenesulfonyl chloride (Fluorochem, Derbyshire, Great Britain) were used without further purification.

### 4.2. General procedures

#### 4.2.1. Preparation of nitriles B

A mixture of 3- or 4-cyanobenzenesulfonyl chloride (605 mg, 3 mmol), the amine (3.3 mmol) and pyridine (736 mg, 9.3 mmol) was stirred overnight at room temperature in abs dichloromethane (40 mL). After removal of the solvent at reduced pressure, nitriles **B** were obtained by flash column chromatography (dichloromethane) and recrystallisation from ethyl acetate/hexane or toluene.

#### 4.2.2. Preparation of amines D and H

To a solution of the Boc-protected amino acid (**C**, 10 mmol) and *N*-methylmorpholine (1.01 g, 10 mmol) in abs THF (20 mL) isobutyl chloroformate (1.37 g, 10 mmol) was added dropwise at −30 °C. After stirring for 15 minutes the amine (10 mmol) was added in one portion. The mixture was stirred and allowed to reach room temperature within 2 h. The reaction was quenched with water (5 mL) and THF was evaporated. The residue was dissolved in ethyl acetate (100 mL), was successively washed with $KHSO_4$ (1 × 25 mL), $NaHCO_3$ (1 × 25 mL) and NaCl (1 × 25 mL) and dried over $Na_2SO_4$. Gaseous HCl was passed through the dried solution for 4 h and the precipitated amine hydrochlorides **D** and **H** were filtered off.

#### 4.2.3. Preparation of amines N

The synthesis of amines **N** was performed as described for amines **D** and **H**, but 4-cyanobenzylamine was employed as hydrochloride. Thus, 2 equivalents of *N*-methylmorpholine were used.

#### 4.2.4. Preparation of amines F and K

Amines **F** and **K** were synthesized according to the preparation of amines **D** and **H** using Cbz-protected amino acids. After extraction the crude product was purified by column chromatography (petroleum ether/ethyl acetate = 6:1) to separate the isobutyl carbamate by-product. The desired product was then eluated from the column using ethyl acetate as the solvent. After reducing the solvent to a volume of 300 mL, the Cbz-protected amine was reduced with 10% Pd/C under a hydrogen atmosphere (3 atm) to afford the amines **F** and **K** after filtration and evaporation of the solvent.

#### 4.2.5. Preparation of nitriles E and I

A solution of 3- or 4-cyanobenzenesulfonyl chloride (605 mg, 3 mmol), the amine hydrochlorides **D** and **H** (3 mmol) and DIPEA (776 mg, 6 mmol) in THF (30 mL) was stirred overnight at room temperature. After removing of the solvent, the crude product was dissolved in ethyl acetate (100 mL) and the mixture was washed with $KHSO_4$ (1 × 50 mL), NaCl (1 × 50 mL) and was dried over $Na_2SO_4$. After evaporation of the solvent, nitriles **E** and **I** were obtained by recrystallisation from ethyl acetate/hexane or ethyl acetate/toluene.

#### 4.2.6. Preparation of nitriles G and L

A solution of 3- or 4-cyanobenzenesulfonyl chloride (605 mg, 3 mmol), the amines **F** or **K** (3 mmol) and DIPEA (388 mg, 3 mmol) in THF (30 mL) was stirred 1 h at room temperature. After evaporation of the solvent and addition of acetic acid (2 mL) the nitriles **G** and **L** were obtained by flash column chromatography (dichloromethane/ethyl acetate = 10:1).

#### 4.2.7. Preparation of nitriles O

A solution of 3- or 4-cyanobenzenesulfonyl chloride (605 mg, 3 mmol), the amine hydrochlorides **N** (3 mmol) and DIPEA (776 mg, 6 mmol) in THF (30 mL) was stirred 1 h at room temperature. After evaporation of the solvent, acetic acid was added (2 mL) and the nitriles **O** were obtained by flash column chromatography (ethyl acetate).

#### 4.2.8. Preparation of benzamidines

A mixture of the corresponding nitrile **B**, **E**, **G**, **I**, or **L**, (1 mmol) hydroxylamine hydrochloride (139 mg, 2 mmol), DIPEA (259 mg, 2 mmol) and abs ethanol (10 mL) was stirred 1 h at 87 °C. Afterwards the solvent was removed under reduced pressure, the residue was taken up in acetonitrile (10 mL) and, if necessary, a minimum amount of acetic acid was added until a nearly complete dissolution was observed. Insoluble solids were filtered off and after addition of acetic anhydride (306 mg, 3 mmol) the solution was stirred 1 h at room temperature. The basic reactants were separated from the desired *O*-acetylamidoximes by flash column chromatography (ethyl acetate/ethanol = 10:1) and after removal of the solvent the crude product was dissolved in acetic acid (20 mL). The reduction was performed with 10% Pd/C under a hydrogen atmosphere (3 atm) at room temperature. After filtration the mixture was evaporated under reduced pressure. The respective benzamidines were obtained as follows.

**4.2.8.1. Benzamidines 1–16.** To the oily residue abs ethyl acetate (−20 °C, 50 mL) was added upon vigorous stirring and the precipitate, which was separated by suction filtration, was dissolved in HCl (0.1 N, 50 mL). After filtration through a syringe filter (cellulose acetate, 0.2 μm) the benzamidines **1**–**16** were obtained after lyophilization.

**4.2.8.2. Benzamidines 17–28.** The oily residue was dissolved in acetic acid (2 mL) and the solution was rapidly injected into stirring ethyl acetate (−20 °C, 200 mL). Gaseous HCl was bubbled through and the precipitate was separated by suction filtration. The solid was dissolved in HCl (0.1 N, 50 mL) and after filtration through a syringe filter (cellulose acetate, 0.2 μm) the benzamidines **17**–**28** were obtained after lyophilization.

**4.2.8.3. Benzamidines 29–32, 33–35 and 36–47.** To the oily residue abs ethyl acetate (−20 °C, 50 mL) was added and after stirring for 1 h the precipitate, which was separated by suction filtration, was dissolved in trifluoroacetic acid (0.1 N, 50 mL). After filtration through a syringe filter (cellulose acetate, 0.2 μm) the benzamidines **29**–**32, 33**–**35** and **36**–**47** were obtained after lyophilization.

**4.2.8.4. Benzamidines 48–59.** The corresponding benzamidines **36**–**47** (100 mg) were stirred in trifluoroacetic acid (6 mL) for 6 h at room temperature. Afterwards, either 50 ml of water were added or the addition of water was stopped when a slight precipitation appeared. Benzamidines **48**–**59** were obtained after filtration through a syringe filter (cellulose acetate, 0.2 μm) followed by lyophilization.

#### 4.2.9. Preparation of bisbenzamidines

A mixture of the corresponding dintrile **O** (1 mmol), hydroxylamine hydrochloride (556 mg, 8 mmol), DIPEA (1.03 g, 8 mmol) and abs ethanol (30 mL) was stirred at 87 °C overnight. Afterwards the solvent was removed in vacuo and the crude product was purified by flash column chromatography (ethyl acetate) after dissolution in acetic acid (3 mL). The residue was taken up in acetonitrile (20 mL) and an appropriate amount of acetic acid was added until a nearly complete solution was observed. Insoluble solids were removed by filtration. Acetic anhydride (1.23 g, 12 mmol) was added and the mixture was stirred at room temperature overnight. After reducing the volume to 20 mL, 10% Pd/C was added and the reduction was carried out under a hydrogen atmosphere (3 atm) at room temperature overnight. After filtration and evaporation of the solvent to a volume of 4 mL, the bisbenzamidinium diacetate was precipitated by dropwise injection of the solution into ethyl acetate (−20 °C, 100 mL). The solid was isolated by suction filtration and

was dissolved in trifluoroacetic acid (0.1 N, 50 mL). The respective bisbenzamidines were obtained as follows.

**4.2.9.1. Bisbenzamidines 60–65.** The bisbenzamidines **60–65** were purified by preparative reversed-phase HPLC (10% MeOH/90% $H_2O$) and were obtained after lyophilization.

**4.2.9.2. Bisbenzamidines 66–73.** The bisbenzamidines **66–73** were purified by preparative reversed-phase HPLC (20% MeOH/80% $H_2O$) and were obtained after lyophilization.

### 4.3. Compounds 1–73 prepared

#### 4.3.1. 4-(*N*-Phenylsulfamoyl)benzamidinium chloride (1)

Yield 48%, mp 126–130 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.04 (tt, $J$ = 7.3 Hz, $J$ = 1.3 Hz, 1H), 7.14 (dd, $J$ = 8.7 Hz, $J$ = 1.3 Hz, 2H), 7.23 (dd, $J$ = 7.3 Hz, $J$ = 8.5 Hz, 2H), 7.94, 7.97 (each d, $J$ = 9.0 Hz, 4H), 9.41, 9.55 (each s, 4H), 10.66 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 120.3, 124.5, 127.1, 129.4, 129.5, 132.3, 137.4, 144.0, 165.1. LC–MS (ESI) purity 96%, $m/z$ 276.4 $[M+H]^+$, (method a). Anal ($C_{13}H_{14}ClN_3O_2S \times 0.1\ H_2O$) C, H, N.

#### 4.3.2. 4-(*N*-(4-Methoxyphenyl)sulfamoyl)benzamidinium chloride (2)

Yield 53%, mp 238–239 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.66 (s, 3H), 6.80 (d, $J$ = 9.2 Hz, 2H), 7.02 (d, $J$ = 8.8 Hz, 2H), 7.88, 7.94 (each d, $J$ = 8.5 Hz, 4H), 9.39, 9.54 (each s, 4H), 10.29 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 55.3, 114.6, 123.6, 127.2, 129.4, 129.8, 132.1, 144.1, 156.8, 165.1. LC–MS (ESI) purity 97%, $m/z$ 306.5 $[M+H]^+$, (method a). Anal ($C_{14}H_{16}ClN_3O_3S \times 0.1\ H_2O$) C, H, N.

#### 4.3.3. 4-(*N*-(3-Methoxyphenyl)sulfamoyl)benzamidinium chloride (3)

Yield 48%, mp 204–205 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.66 (s, 3H), 6.61 (ddd, $J$ = 8.1 Hz, $J$ = 2.2 Hz, $J$ = 1.0 Hz, 1H), 6.71–6.75 (m, 2H), 7.13 (dd, $J$ = 8.2 Hz, $J$ = 8.4 Hz, 1H), 7.95, 7.98 (each d, $J$ = 8.8 Hz, 4H), 9.42, 9.55 (each s, 4H), 10.68 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 55.2, 106.0, 109.5, 112.1, 127.1, 129.5, 130.3, 132.5, 138.6, 144.0, 159.8, 165.1. LC–MS (ESI) purity 94%, $m/z$ 306.4 $[M+H]^+$, (method a). Anal ($C_{14}H_{16}ClN_3O_3S \times 0.1\ H_2O$) C, H, N.

#### 4.3.4. 4-(*N*-(3,4-Dimethoxyphenyl)sulfamoyl)benzamidinium chloride (4)

Yield 64%, mp 137–141 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.63, 3.65 (each s, 6H), 6.60 (dd, $J$ = 8.5 Hz, $J$ = 2.6 Hz, 1H), 6.73 (d, $J$ = 2.5 Hz, 1H), 6.79 (d, $J$ = 8.8 Hz, 1H), 7.91, 7.94 (each d, $J$ = 8.8 Hz, 4H), 9.38, 9.54 (each s, 4H), 10.29 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 55.6, 55.8, 106.6, 112.3, 113.7, 127.2, 129.4, 130.2, 132.1, 144.0, 146.4, 149.0, 165.1. LC–MS (ESI) purity 99%, $m/z$ 336.0 $[M+H]^+$, (method a). Anal ($C_{15}H_{18}ClN_3O_4S \times 0.4\ H_2O$) C, H, N.

#### 4.3.5. 4-(*N*-Benzylsulfamoyl)benzamidinium chloride (5)

Yield 13%, mp 238–239 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 4.03 (d, $J$ = 6.3 Hz, 2H), 7.20–7.31 (m, 5H), 7.97–8.02 (m, 4H), 8.51 (t, $J$ = 6.3 Hz, 1H), 9.44, 9.60 (each s, 4H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 46.3, 127.4, 126.9, 127.7, 128.4, 129.4, 131.7, 137.5, 145.4, 165.0. LC–MS (ESI) purity 99%, $m/z$ 290.4 $[M+H]^+$, (method a). Anal ($C_{14}H_{16}ClN_3O_2S$) C, H, N.

#### 4.3.6. 4-(*N*-(4-Methoxybenzyl)sulfamoyl)benzamidinium chloride (6)

Yield 47%, mp 107–110 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.70 (s, 3H), 3.95 (s, 2H), 6.83 (d, $J$ = 8.5 Hz, 2H), 7.13 (d, $J$ = 8.5 Hz, 2H), 7.96, 7.99 (each d, $J$ = 9.0 Hz, 4H), 8.41 (s, 1H), 9.50 (s, 4H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 45.8, 55.2, 113.8, 126.9, 129.1, 129.3, 129.4, 131.6, 145.4, 158.7, 165.0. LC–MS (ESI) purity 97%, $m/z$ 320.1 $[M+H]^+$, (method a). Anal ($C_{15}H_{18}ClN_3O_3S \times 0.5\ H_2O$) C, H, N.

#### 4.3.7. 4-(*N*-(3-Methoxybenzyl)sulfamoyl)benzamidinium chloride (7)

Yield 32%, mp 186–188 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.69 (s, 3H), 4.01 (d, $J$ = 6.3 Hz, 2H), 6.77–6.82 (m, 3H), 7.17–7.21 (m, 1H), 7.98, 8.01 (each d, $J$ = 8.2 Hz, 4H), 8.50 (t, $J$ = 6.3 Hz, 1H), 9.43, 9.59 (each s, 4H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 46.2, 55.2, 112.9, 113.3, 119.8, 126.9, 129.3, 129.5, 139.1, 145.4, 159.4, 165.0. LC–MS (ESI) purity 97%, $m/z$ 320.1 $[M+H]^+$, (method a). Anal ($C_{15}H_{18}ClN_3O_3S \times 0.3\ H_2O$) C, H, N.

#### 4.3.8. 4-(*N*-(3,4-Dimethoxybenzyl)sulfamoyl)benzamidinium chloride (8)

Yield 54%, mp 124–127 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.67, 3.69 (each s, 6H), 3.96 (d, $J$ = 6.3 Hz, 2H), 6.72 (dd, $J$ = 8.2 Hz, $J$ = 1.9 Hz, 1H), 6.80 (d, $J$ = 1.9 Hz, 1H), 6.82 (d, $J$ = 8.2 Hz, 1H), 7.97, 7.99 (each d, $J$ = 8.8 Hz, 4H), 8.41 (t, $J$ = 6.2 Hz, 1H), 9.41, 9.57 (each s, 4H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 46.2, 55.6, 55.7, 111.7, 111.8, 120.0, 126.9, 129.3, 129.7, 131.6, 145.5, 148.2, 148.7, 164.9. LC–MS (ESI) purity 95%, $m/z$ 350.3 $[M+H]^+$, (method a). Anal ($C_{16}H_{20}ClN_3O_4S \times 0.7\ H_2O$) C, H, N.

#### 4.3.9. 3-(*N*-Phenylsulfamoyl)benzamidinium chloride (9)

Yield 75%, mp 262–263 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.02–7.05 (m, 1H), 7.11–7.13 (m, 2H), 7.22 (dd, $J$ = 7.5 Hz, $J$ = 7.3 Hz, 2H), 7.77 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 8.00–8.06 (m, 2H), 8.26 (dd, $J$ = 1.7 Hz, $J$ = 1.6 Hz, 1H), 9.40, 9.61 (each s, 4H), 10.55 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 120.6, 124.6, 126.7, 129.3, 129.3, 130.2, 131.3, 132.7, 137.3, 164.9. LC–MS (ESI) purity 95%, $m/z$ 276.3 $[M+H]^+$, (method a). Anal ($C_{13}H_{14}ClN_3O_2S$) C, H, N.

#### 4.3.10. 3-(*N*-(4-Methoxyphenyl)sulfamoyl)benzamidinium chloride (10)

Yield 58%, mp 230–232 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.65 (s, 3H), 6.79 (d, $J$ = 9.1 Hz, 2H), 7.01 (d, $J$ = 9.2 Hz, 2H), 7.76 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 8.02, 7.96 (d, $J$ = 7.9 Hz, 2H), 8.22 (dd, 1H, $J$ = 1.6 Hz, $J$ = 1.6 Hz), 9.39, 9.60 (each s, 4H), 8.33 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 55.3, 114.5, 124.0, 126.7, 129.3, 129.7, 130.1, 131.4, 132.5, 140.5, 156.9, 165.0. LC–MS (ESI) purity 95%, $m/z$ 306.5 $[M+H]^+$, (method a). Anal ($C_{14}H_{16}ClN_3O_3S$) C, H, N.

#### 4.3.11. 3-(*N*-(3-Methoxyphenyl)sulfamoyl)benzamidinium chloride (11)

Yield 66%, mp 184–186 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.65 (s, 3H), 6.61 (ddd, $J$ = 8.4 Hz, $J$ = 2.5 Hz, $J$ = 1.0 Hz, 1H), 6.70–6.74 (m, 2H), 7.12 (dd, $J$ = 8.2 Hz, $J$ = 8.2 Hz, 1H), 7.78 (dd, $J$ = 8.2 Hz, $J$ = 7.9 Hz, 1H), 8.01–8.07 (m, 2H), 8.27 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 9.40, 9.61 (each s, 4H), 10.54 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 55.2, 106.5, 109.7, 112.6, 126.7, 129.4, 130.2, 130.2, 131.3, 132.7, 138.6, 140.5, 159.8, 165.0. LC–MS (ESI) purity 94%, $m/z$ 306.5 $[M+H]^+$, (method a). Anal ($C_{14}H_{16}ClN_3O_3S \times 0.1\ H_2O$) C, H, N.

#### 4.3.12. 3-(*N*-(3,4-Dimethoxyphenyl)sulfamoyl)benzamidinium chloride (12)

Yield 51%, mp 205–206 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.61, 3.65 (each s, 6H), 6.59 (dd, $J$ = 8.8, Hz, $J$ = 2.6 Hz, 1H), 6.71 (d, $J$ = 2.6 Hz, 1H), 6.78 (d, $J$ = 8.8 Hz, 1H), 7.77 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.97–8.03 (m, 2H, 4-H), 8.22 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 9.35, 9.59 (each s, 4H), 10.15 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 55.6, 55.8, 107.3, 112.2, 114.3, 126.7, 129.3, 130.1, 130.1, 131.5, 132.5, 140.4, 146.6, 148.9, 164.9. LC–MS (ESI) purity 95%, $m/z$ 336.0 $[M+H]^+$, (method a). Anal ($C_{15}H_{18}ClN_3O_4S$) C, H, N.

#### 4.3.13. 3-(*N*-Benzylsulfamoyl)benzamidinium chloride (13)

Yield 41%, mp 207–209 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 4.03 (d, 2H, $J$ = 6.3 Hz), 7.17–7.30 (m, 5H), 7.79 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 8.03–8.10 (m, 2H), 8.23 (dd, $J$ = 1.7 Hz, $J$ = 1.6 Hz, 1H), 8.43 (t, $J$ = 6.3 Hz, 1H), 9.42, 9.63 (each s, 4H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 126.5, 127.3, 127.8, 128.3, 129.1, 130.2, 131.3, 132.0, 137.6, 141.7, 165.0. LC–MS (ESI) purity 97%, $m/z$ 290.3 $[M+H]^+$, (method a). Anal ($C_{14}H_{16}ClN_3O_2S$) C, H, N.

#### 4.3.14. 3-(*N*-(4-Methoxybenzyl)sulfamoyl)benzamidinium chloride (14)

Yield 63%, mp 186–187 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.70 (s, 3H), 3.95 (d, $J$ = 6.0 Hz, 2H), 6.80 (d, $J$ = 8.8 Hz, 2H), 7.13 (d, $J$ = 8.8 Hz, 2H), 7.79 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 8.03–8.09 (m, 2H), 8.22 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 8.33 (t, $J$ = 6.3 Hz, 1H), 9.43, 9.63 (each s, 4H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 45.8, 55.2, 113.8, 126.5, 129.0, 129.4, 129.2, 130.2, 131.3, 132.0, 141.8, 158.6, 164.9. LC–MS (ESI) purity 96%, $m/z$ 320.3 $[M+H]^+$, (method a). Anal ($C_{15}H_{18}ClN_3O_2S$) C, H, N.

#### 4.3.15. 3-(*N*-(3-Methoxybenzyl)sulfamoyl)benzamidinium chloride (15)

Yield 80%, mp 104–109 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.68 (s, 3H), 4.01 (d, $J$ = 6.3 Hz, 2H), 6.74–6.82 (m, 3H), 7.79 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 8.01–8.09 (m, 2H), 8.20–8.24 (m, 1H), 8.41 (t, $J$ = 6.3 Hz, 1H), 9.39, 9.60 (each s, 4H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 46.2, 55.1, 112.8, 113.3, 119.9, 126.5, 129.0, 129.4, 130.2, 131.3, 131.9, 139.1, 141.8, 159.3, 164.9. LC–MS (ESI) purity 98%, $m/z$ 320.0 $[M+H]^+$, (method a). Anal ($C_{15}H_{18}ClN_3O_3S \times 0.9\ H_2O$) C, H, N.

#### 4.3.16. 3-(*N*-(3,4-Dimethoxybenzyl)sulfamoyl)benzamidinium chloride (16)

Yield 58%, mp 122–131 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.64, 3.69 (each s, 6H), 3.96 (s, 2H), 6.73 (dd, $J$ = 8.2 Hz, $J$ = 1.9 Hz, 1H), 6.80 (d, $J$ = 1.9 Hz, 1H), 6.82 (d, $J$ = 8.2 Hz, 1H), 7.77 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 8.02–8.07 (m, 2H), 8.23 (dd, $J$ = 1.6 Hz, $J$ = 1.7 Hz, 1H), 8.33 (s, 1H), 9.45, 9.57 (each s, 4H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 46.2, 55.5, 55.7, 111.6, 111.8, 120.1, 126.5, 128.9, 129.7, 130.1, 131.3, 131.9, 141.9, 148.2, 148.7, 164.8. LC–MS (ESI) purity 96%, $m/z$ 350.1 $[M+H]^+$. Anal ($C_{16}H_{20}ClN_3O_4S \times 0.5\ H_2O$) C, H, N.

#### 4.3.17. 4-(*N*-(2-(3,4-Dimethoxyphenylamino)-2-oxoethyl)-sulfamoyl)benzamidinium chloride (17)

Yield 47%, mp 140–150 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.68, 3.69 (each s, 6H), 3.71 (d, $J$ = 6.0 Hz, 2H), 6.84 (d, $J$ = 8.8 Hz, 1H), 7.02 (dd, $J$ = 8.7 Hz, $J$ = 2.5 Hz, 1H), 7.18 (d, $J$ = 2.5 Hz, 1H), 7.99, 8.02 (each d, $J$ = 8.8 Hz, 4H), 8.42 (t, $J$ = 6.0 Hz, 1H), 9.37, 9.56 (each s, 4H), 10.00 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 45.9, 55.6, 55.9, 104.8, 111.5, 112.3, 127.1, 129.3, 131.7, 132.3, 145.2, 145.3, 148.7, 165.0, 165.8. LC–MS (ESI) purity 96%, $m/z$ 393.3 $[M+H]^+$, (method a). Anal ($C_{17}H_{21}ClN_4O_5S \times 1.0\ H_2O$) C, H, N.

#### 4.3.18. 4-(*N*-(3-(3,4-Dimethoxyphenylamino)-3-oxopropyl)-sulfamoyl)benzamidinium chloride (18)

Yield 51%, mp 150–170 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 2.45–2.50 (m, 2H), 3.03–3.07 (m, 2H), 3.69, 3.69 (each s, 6H), 6.84 (d, $J$ = 8.9 Hz, 1H), 7.07 (dd, $J$ = 8.7 Hz, $J$ = 2.5 Hz, 1H), 7.31 (d, $J$ = 2.3 Hz, 1H), 8.00 (s, 4H), 8.10 (t, $J$ = 5.7 Hz, 1H), 9.43, 9.60 (each s, 4H), 9.96 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 36.5, 39.1, 55.5, 55.9, 104.6, 111.2, 112.2, 127.0, 129.4, 131.9, 132.9, 144.8, 144.9, 148.6, 165.2, 168.3. LC–MS (ESI) purity 96%, $m/z$ 407.3 $[M+H]^+$, (method a). Anal ($C_{18}H_{23}ClN_4O_5S \times 1.5\ H_2O$) C, H, N.

#### 4.3.19. 4-(*N*-(2-(3,4-Dimethoxybenzylamino)-2-oxoethyl)-sulfamoyl)benzamidinium chloride (19)

Yield 8%, mp 164–166 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.53 (d, $J$ = 6.0 Hz, 2H), 3.71, 3.72 (each s, 6H), 4.15 (d, $J$ = 6.0 Hz, 2H), 6.72 (dd, $J$ = 8.2 Hz, $J$ = 1.9 Hz, 1H), 6.84–6.86 (m, 2H), 7.96, 8.00 (each d, $J$ = 8.7 Hz, 4H), 8.31–8.35 (m, 2H), 9.42, 9.46 (each s, 4H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 42.0, 45.3, 55.6, 55.8, 111.6, 111.9, 119.6, 127.1, 129.3, 131.6, 132.0, 145.1, 148.0, 148.8, 165.1, 167.4. LC–MS (ESI) purity 95%, $m/z$ 407.1 $[M+H]^+$, (method a). Anal ($C_{18}H_{23}ClN_4O_5S \times 1.3\ H_2O$) C, H, N.

#### 4.3.20. 4-(*N*-(4-(3,4-Dimethoxyphenylamino)-4-oxobutyl)-sulfamoyl)benzamidinium chloride (20)

Yield 65%, mp 265 °C (Zers.). $^1$H NMR (DMSO-$d_6$): $\delta$ 1.70 (tt, $J$ = 7.3 Hz, $J$ = 7.3 Hz, 2H), 2.29 (t, $J$ = 7.3 Hz, 2H), 2.80–2.84 (m, 2H), 3.69, 3.69 (each s, 6H), 6.84 (d, $J$ = 8.5 Hz, 1H), 7.07 (dd, $J$ = 8.7 Hz, $J$ = 2.5 Hz, 1H), 7.29 (d, $J$ = 2.6 Hz, 1H), 7.99 (s, 4H), 8.02 (t, $J$ = 5.7 Hz, 1H), 9.37, 9.56 (each s, 4H), 9.85 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 25.3, 33.3, 42.4, 55.5, 55.9, 104.6, 111.1, 112.3, 126.9, 129.5, 131.8, 133.1, 144.8, 145.1, 148.6, 165.1, 170.2. LC–MS (ESI) purity 98%, $m/z$ 421.4 $[M+H]^+$, (method a). Anal ($C_{19}H_{25}ClN_4O_5S \times 0.4\ H_2O$) C, H, N.

#### 4.3.21. 4-(*N*-(3-(3,4-Dimethoxybenzylamino)-3-oxopropyl) sulfamoyl)benzamidinium chloride (21)

Yield 45%, mp 164–166 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 2.34 (t, $J$ = 7.3 Hz, 2H), 2.96–3.00 (m, 2H), 3.70, 3.71 (each s, 6H), 4.14 (d, $J$ = 5.7 Hz, 2H), 6.74 (dd, $J$ = 8.2 Hz, $J$ = 2.2 Hz, 1H), 6.84 (d, $J$ = 1.9 Hz, 1H), 6.85 (d, $J$ = 8.5 Hz), 8.00, 8.00 (each s, 4H), 8.04 (t, $J$ = 6.0 Hz, 1H), 8.38 (t, $J$ = 5.7 Hz, 1H), 9.44, 9.61 (each s, 4H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 35.7, 39.4, 42.0, 55.6, 55.8, 111.6, 111.9, 119.6, 127.0, 129.5, 131.9, 132.0, 144.8, 147.9, 148.8, 165.2, 169.6. LC–MS (ESI) purity 97%, $m/z$ 421.5 $[M+H]^+$, (method a). Anal ($C_{19}H_{25}ClN_4O_5S \times 1.9\ H_2O$) C, H, N.

#### 4.3.22. 4-(*N*-(4-(3,4-Dimethoxybenzylamino)-4-oxobutyl)-sulfamoyl)benzamidinium chloride (22)

Yield 48%, mp 134–140 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.64 (tt, $J$ = 7.3 Hz, $J$ = 7.3 Hz, 2H), 2.12 (t, $J$ = 7.3 Hz, 2H), 2.76–2.80 (m, 2H), 3.70, 3.70 (each s, 6H), 4.14 (d, $J$ = 5.7 Hz, 2H), 6.72 (dd, $J$ = 8.2 Hz, $J$ = 1.9 Hz, 1H), 6.83 (d, $J$ = 2.2 Hz, 1H), 6.85 (d, $J$ = 8.2 Hz), 7.98, 8.01 (each d, $J$ = 8.7 Hz, 4H), 7.97–8.02 (m, 1H), 8.29 (t, $J$ = 5.7 Hz, 1H), 9.45, 9.61 (each s, 4H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 25.6, 32.5, 41.9, 42.4, 55.6, 55.8, 111.6, 111.9, 119.5, 126.9, 129.5, 131.8, 132.2, 145.2, 147.9, 148.8, 165.2, 171.5. LC–MS (ESI) purity 99%, $m/z$ 435.6 $[M+H]^+$, (method a). Anal ($C_{20}H_{27}ClN_4O_5S \times 1.5\ H_2O$) C, H, N.

#### 4.3.23. 3-(*N*-(2-(3,4-Dimethoxyphenylamino)-2-oxoethyl)-sulfamoyl)benzamidinium chloride (23)

Yield 30%, mp 98–104 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.68, 3.69 (each s, 6H), 3.71 (d, $J$ = 5.7 Hz, 2H), 6.84 (d, $J$ = 8.8 Hz, 1H), 7.03 (dd, $J$ = 8.7 Hz, $J$ = 2.5 Hz, 1H), 7.20 (d, $J$ = 2.6 Hz, 1H), 7.81 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 8.01–8.03 (m, 1H), 8.13–8.15 (m, 1H), 8.26–8.31 (m, 2H, 2-H), 9.35, 9.62 (each s, 4H), 10.04 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 45.9, 55.5, 55.9, 104.7, 111.4, 112.2, 126.6, 129.0, 130.2, 131.5, 132.2, 132.3, 141.4, 145.1, 148.7, 164.9, 165.9. LC–MS (ESI) purity 96%, $m/z$ 393.3 $[M+H]^+$, (method a). Anal ($C_{17}H_{21}ClN_4O_5S \times 2.0\ H_2O$) C, H, N.

#### 4.3.24. 3-(*N*-(3-(3,4-Dimethoxyphenylamino)-3-oxopropyl)-sulfamoyl)benzamidinium chloride (24)

Yield 63%, mp 92–100 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 2.45–2.50 (m, 2H), 3.04–3.08 (m, 2H), 3.69, 3.69 (each s, 6H), 6.84 (d, $J$ = 8.9 Hz, 1H), 7.06 (dd, $J$ = 8.7 Hz, $J$ = 2.6 Hz, 1H), 7.30 (d, $J$ = 2.5 Hz, 1H), 7.83 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 8.00 (t, $J$ = 5.7 Hz, 1H), 8.04–8.06 (m, 1H), 8.11–8.13 (m, 1H), 8.25 (dd, $J$ = 1.9 Hz, $J$ = 1.9 Hz, 1H), 9.38, 9.62 (each s, 4H), 9.93 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 36.6, 39.1, 55.5, 55.9, 104.6, 111.2, 112.2, 126.5, 129.3, 130.2, 131.3, 132.1, 132.9, 141.3, 144.9, 148.6, 165.1, 168.3. LC–

MS (ESI) purity 95%, *m/z* 407.3 $[M+H]^+$, (method a). Anal ($C_{18}H_{23}ClN_4O_5S \times 2.2\ H_2O$) C, H, N.

#### 4.3.25. 3-(*N*-(2-(3,4-Dimethoxybenzylamino)-2-oxoethyl)-sulfamoyl)benzamidinium chloride (25)

Yield 29%, mp 120–124 °C. $^1H$ NMR (DMSO-$d_6$): $\delta$ 3.53 (d, $J$ = 5.7 Hz, 2H), 3.71, 3.71 (each s, 6H), 4.11 (d, $J$ = 6.0 Hz, 2H), 6.71 (dd, $J$ = 8.2 Hz, $J$ = 1.9 Hz, 1H), 6.84 (d, $J$ = 1.9 Hz, 1H), 6.84 (d, $J$ = 8.2 Hz, 1H), 7.80 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 8.03–8.05 (m, 1H), 8.11–8.13 (m, 1H), 8.25 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 8.29 (t, $J$ = 6.3 Hz, 1H), 8.41 (t, $J$ = 6.0 Hz), 9.41, 9.63 (each s, 4H). APT $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 42.0, 45.4, 55.6, 55.8, 111.6, 111.9, 119.4, 126.6, 129.1, 130.2, 131.5, 132.2, 131.6, 141.2, 147.9, 148.8, 165.0, 167.6. LC–MS (ESI) purity 95%, *m/z* 407.1 $[M+H]^+$, (method a). Anal ($C_{17}H_{21}ClN_4O_5S \times 1.6\ H_2O$) C, H, N.

#### 4.3.26. 3-(*N*-(4-(3,4-Dimethoxyphenylamino)-4-oxobutyl)-sulfamoyl)benzamidinium chloride (26)

Yield 65%, mp 123–125 °C. $^1H$ NMR (DMSO-$d_6$): $\delta$ 1.69 (tt, $J$ = 7.3 Hz, $J$ = 7.3 Hz, 2H), 2.29 (t, $J$ = 7.3 Hz, 2H), 2.79–2.82 (m, 2H), 3.69, 3.69 (each s, 6H), 6.84 (d, $J$ = 8.9 Hz, 1H), 7.07 (dd, $J$ = 8.7 Hz, $J$ = 2.3 Hz, 1H), 7.30 (d, $J$ = 2.3 Hz, 1H), 7.82 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.94 (t, $J$ = 5.7 Hz, 1H), 8.04–8.06 (m, 1H), 8.10–8.12 (m, 1H), 8.25 (s, 1H), 9.41, 9.64 (each s, 4H), 9.88 (s, 1H). APT $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 25.3, 33.3, 42.3, 55.5, 55.9, 104.6, 111.1, 112.3, 126.5, 129.3, 130.2, 131.3, 132.1, 133.1, 141.4, 144.8, 148.6, 165.1, 170.3. LC–MS (ESI) purity 99%, *m/z* 421.3 $[M+H]^+$, (method a). Anal ($C_{19}H_{25}ClN_4O_5S \times 1.1\ H_2O$) C, H, N.

#### 4.3.27. 3-(*N*-(3-(3,4-Dimethoxybenzylamino)-3-oxopropyl)-sulfamoyl)benzamidinium chloride (27)

Yield 53%, mp 132–138 °C. $^1H$ NMR (DMSO-$d_6$): $\delta$ 2.33 (t, $J$ = 7.3 Hz, 2H), 2.97–3.01 (m, 2H), 3.70, 3.71 (each s, 6H), 4.14 (d, $J$ = 5.7 Hz, 2H), 6.73 (dd, $J$ = 8.1 Hz, $J$ = 1.9 Hz, 1H), 6.83 (d, $J$ = 1.9 Hz, 1H), 6.85 (d, $J$ = 8.2 Hz, 1H), 7.82 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.95 (t, $J$ = 5.7 Hz, 1H), 8.05–8.07 (m, 1H), 8.10–8.12 (m, 1H), 8.25 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 8.38 (t, $J$ = 5.7 Hz), 9.44, 9.65 (each s, 4H). APT $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 35.6, 39.4, 42.0, 55.6, 55.8, 111.6, 111.9, 119.5, 126.5, 129.3, 130.2, 131.3, 132.2, 131.9, 141.3, 147.9, 148.8, 165.1, 169.5. LC–MS (ESI) purity 97%, *m/z* 421.3 $[M+H]^+$, (method a). Anal ($C_{19}H_{25}ClN_4O_5S \times 2.0\ H_2O$) C, H, N.

#### 4.3.28. 3-(*N*-(4-(3,4-Dimethoxybenzylamino)-4-oxobutyl)-sulfamoyl)benzamidinium chloride (28)

Yield 62%, mp 114–124 °C. $^1H$ NMR (DMSO-$d_6$): $\delta$ 1.62 (tt, $J$ = 7.3 Hz, $J$ = 7.3 Hz, 2H), 2.13 (t, $J$ = 7.6 Hz, 2H), 2.75–2.78 (m, 2H), 3.70, 3.70 (each s, 6H), 4.13 (d, $J$ = 5.7 Hz, 2H), 6.72 (dd, $J$ = 8.0 Hz, $J$ = 1.9 Hz, 1H), 6.82 (d, $J$ = 1.9 Hz, 1H), 6.84 (d, $J$ = 8.2 Hz, 1H), 7.82 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.92 (t, $J$ = 5.4 Hz, 1H), 8.05–8.07 (m, 1H), 8.08–8.10 (m, 1H), 8.25 (dd, $J$ = 1.9 Hz, $J$ = 1.9 Hz, 1H), 8.30 (t, $J$ = 5.7 Hz), 9.45, 9.66 (each s, 4H). APT $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 25.6, 32.5, 41.9, 42.4, 55.6, 55.8, 111.5, 112.0, 119.4, 126.5, 129.2, 130.2, 131.2, 132.1, 132.2, 141.5, 147.9, 148.8, 165.1, 171.6. LC–MS (ESI) purity 97%, *m/z* 435.4 $[M+H]^+$, (method a). Anal ($C_{20}H_{27}ClN_4O_5S \times 1.8\ H_2O$) C, H, N.

#### 4.3.29. 3-(*N*-(2-Phenylamino)-2-oxoethyl)-*N*-sulfamoyl)-benzamidinium trifluoroacetate (29)

Yield 46%, mp 87–99 °C. $^1H$ NMR (DMSO-$d_6$): $\delta$ 3.75 (d, $J$ = 6.0 Hz, 2H), 7.03 (tt, $J$ = 8.5 Hz, $J$ = 1.0 Hz), 7.27 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 2H), 7.44 (dd, $J$ = 8.5 Hz, $J$ = 1.3 Hz, 2H), 7.83 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 8.01–8.03 (m, 1H), 8.14–8.17 (m, 1H), 8.21 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 8.28 (t, $J$ = 6.0 Hz, 1H), 9.41, 9.49 (each s, 4H), 9.97 (s, 1H). APT $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 45.9, 119.4, 123.6, 126.4, 128.9, 129.3, 130.2, 131.5, 132.1, 138.6, 141.6, 158.7 (q, $J$ = 31.9 Hz), 165.0, 166.3. LC–MS (ESI) purity 98%, *m/z* 333.1 $[M+H]^+$, (method b). Anal ($C_{17}H_{17}F_3N_4O_5S \times 1.0\ H_2O$) C, H, N.

#### 4.3.30. 3-(*N*-(2-(Naphthalen-2-ylamino)-2-oxoethyl)sulfamoyl) benzamidinium trifluoroacetate (30)

Yield 64%, mp 216–220 °C. $^1H$ NMR (DMSO-$d_6$): $\delta$ 3.83 (s, 2H), 7.40 (ddd, $J$ = 7.4 Hz, $J$ = 7.4 Hz, $J$ = 1.0 Hz, 1H), 7.46 (ddd, $J$ = 7.4 Hz, $J$ = 7.4 Hz, $J$ = 1.3 Hz, 1H), 7.52 (dd, $J$ = 8.8 Hz, $J$ = 2.2 Hz, 1H), 7.78–7.86 (m, 4H), 8.01–8.03 (m, 1H), 8.14 (d, $J$ = 1.6 Hz), 8.17–8.19 (m, 1H), 8.24 (dd, $J$ = 1.9 Hz, $J$ = 1.9 Hz, 1H), 9.24 (s, 4H). APT $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 46.0, 115.6, 120.0, 126.4, 124.9, 126.6, 127.4, 127.6, 128.6, 130.0, 130.2, 131.5, 132.1, 133.4, 136.2, 141.6, 158.7 (q, $J$ = 31.9 Hz), 165.0, 166.7. LC–MS (ESI) purity 95%, *m/z* 383.3 $[M+H]^+$, (method b). Anal ($C_{21}H_{19}F_3N_4O_5S$) C, H, N.

#### 4.3.31. 3-(*N*-(2-Cyclohexylamino)-2-oxoethyl)-*N*-sulfamoyl)-benzamidinium trifluoroacetate (31)

Yield 73%, mp 194–199 °C. $^1H$ NMR (DMSO-$d_6$): $\delta$ 1.04–1.25 (m, 5H), 1.49–1.63 (m, 5H), 3.36–3.43 (m, 1H), 3.49 (d, $J$ = 6.3 Hz, 2H), 7.68 (d, $J$ = 7.9 Hz, 1H), 7.82 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 8.01–8.03 (m, 1H), 8.09–8.12 (m, 2H, 4-H), 8.16 (dd, $J$ = 1.9 Hz, $J$ = 1.9 Hz, 1H), 9.51 (s, 4H). APT $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 24.5, 25.3, 32.3, 45.3, 47.7, 126.4, 129.3, 130.2, 131.5, 132.1, 141.5, 158.9 (q, $J$ = 31.9 Hz), 165.1, 166.3. LC–MS (ESI) purity 99%, *m/z* 339.4 $[M+H]^+$. Anal ($C_{17}H_{23}F_3N_4O_5S \times 0.7\ H_2O$) C, H, N.

#### 4.3.32. 3-(*N*-(2-Isopropylamino)-2-oxoethyl)-*N*-sulfamoyl)benzamidinium trifluoroacetate (32)

Yield 64%, mp 80–90 °C. $^1H$ NMR (DMSO-$d_6$): $\delta$ 0.96 (d, $J$ = 6.7 Hz, 6H), 3.47 (d, $J$ = 6.3 Hz, 2H), 3–66–3.73 (m, 1H), 7.67 (d, $J$ = 7.9 Hz, 1H), 7.82 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 8.01–8.03 (m, 1H), 8.09–8.12 (m, 2H), 8.17 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 9.42, 9.49 (each s, 4H). APT $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 22.3, 40.6, 45.3, 126.4, 129.3, 130.2, 131.5, 132.1, 141.5, 158.7 (q, $J$ = 31.9 Hz), 165.0, 166.3. LC–MS (ESI) purity 97%, *m/z* 299.4 $[M+H]^+$, (method b). Anal ($C_{14}H_{19}F_3N_4O_5S \times 0.9\ H_2O$) C, H, N.

#### 4.3.33. 3-(2-(3,4-Dimethoxyphenylsulfonamido)acetamido)-benzamidinium trifluoroacetate (33)

Yield 30%, mp 209–212 °C. $^1H$ NMR (DMSO-$d_6$): $\delta$ 3.65 (d, $J$ = 6.3 Hz, 2H), 3.80 (s, 6H), 7.35 (d, $J$ = 1.9 Hz, 1H), 7.39 (dd, $J$ = 8.2 Hz, $J$ = 2.2 Hz, 1H), 7.42–7.44 (m, 1H), 7.53 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.75–7.78 (m, 1H), 7.93 (t, $J$ = 6.0 Hz, 1H), 7.97 (dd, $J$ = 1.9 Hz, $J$ = 1.9 Hz, 1H), 9.15, 9.29 (each s, 4H), 10.23 (s, 1H). APT $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 46.1, 109.8, 111.3, 118.5, 120.5, 123.1, 124.1, 129.4, 129.7, 131.9, 139.2, 148.8, 152.2, 158.5 (q, $J$ = 32.8 Hz), 166.2, 167.2. LC–MS (ESI) purity 96%, *m/z* 393.4 $[M+H]^+$, (method b). Anal ($C_{19}H_{21}F_3N_4O_7S$) C, H, N.

#### 4.3.34. 3-(2-(Naphthalene-2-sulfonamido)acetamido)benz-amidinium trifluoroacetate (34)

Yield 44%, mp 249–253 °C. $^1H$ NMR (DMSO-$d_6$): $\delta$ 3.74 (d, $J$ = 6.0 Hz, 2H), 7.39–7.41 (m, 1H), 7.49 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.63–7.71 (m, 3H), 7.86 (dd, $J$ = 8.9 Hz, $J$ = 1.9 Hz, 1H), 7.92 (dd, $J$ = 1.9 Hz, $J$ = 1.9 Hz, 1H), 8.02 (d, $J$ = 8.2 Hz, 1H), 8.11–8.13 (m, 2H), 8.22 (t, $J$ = 6.0 Hz, 1H), 8.45 (d, $J$ = 1.6 Hz, 1H), 9.17, 9.27 (each s, 4H), 10.26 (s, 1H). APT $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 46.0, 118.5, 122.5, 123.1, 124.0, 127.5, 127.7, 127.9, 128.9, 129.3, 129.4, 129.3, 131.8, 134.3, 137.6, 139.2, 158.6 (q, $J$ = 31.0 Hz), 166.2, 167.1. LC–MS (ESI) purity 95%, *m/z* 383.2 $[M+H]^+$, (method b). Anal ($C_{21}H_{19}F_3N_4O_5S \times 0.4\ H_2O$) C, H, N.

#### 4.3.35. 3-(2-(Naphthalene-1-sulfonamido)acetamido)benz-amidinium trifluoroacetate (35)

Yield 20%, mp 237–241 °C. $^1H$ NMR (DMSO-$d_6$): $\delta$ 3.75 (d, $J$ = 6.3 Hz, 2H), 7.40–7.42 (m, 1H), 7.51 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz,

1H), 7.61–7.73 (m, 4H), 7.92 (dd, $J$ = 1.9 Hz, $J$ = 1.9 Hz, 1H), 8.07 (dd, $J$ = 7.9 Hz, $J$ = 0.6 Hz, 1H), 8.16 (dd, $J$ = 7.6 Hz, $J$ = 1.0 Hz, 1H), 8.20 (d, $J$ = 8.6 Hz, 1H), 8.49 (t, $J$ = 6.3 Hz, 1H), 8.68 (dd, $J$ = 1.6 Hz, $J$ = 1.0 Hz, 1H), 9.21, 9.29 (each s, 4H), 10.23 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 45.8, 118.4, 123.1, 123.9, 124.6, 125.0, 127.0, 128.0, 128.4, 129.0, 127.7, 129.4, 129.7, 133.9, 134.0, 135.9, 139.1, 158.7 (q, $J$ = 31.0 Hz), 166.3, 167.3. LC–MS (ESI) purity 96%, $m/z$ 383.2 $[M+H]^+$, (method b). Anal ($C_{21}H_{19}F_3N_4O_5S \times 0.5\ H_2O$) C, H, N.

#### 4.3.36. 4-(*N*-(2-(4-(*tert*-Butoxycarbonyl)phenylamino)-2-oxoethyl)sulfamoyl)benzamidinium trifluoroacetate (36)

Yield 23%, mp >250 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.52 (s, 9H), 3.80 (d, $J$ = 6.0 Hz, 2H), 7.59 (d, $J$ = 8.8 Hz, 2H), 7.82 (d, $J$ = 8.9 Hz, 2H), 7.97, 8.02 (each d, $J$ = 8.5 Hz, 4H), 8.43 (t, $J$ = 6.0 Hz, 1H), 9.45, 9.46 (each s, 4H), 10.31 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 28.0, 45.9, 80.5, 118.6, 126.1, 127.0, 129.2, 130.2, 132.0, 142.7, 145.3, 158.8 (q, $J$ = 31.3 Hz), 164.7, 165.0, 166.9. LC–MS (ESI) purity 99%, $m/z$ 433.3 $[M+H]^+$, (method c). Anal ($C_{22}H_{25}F_3N_4O_7S \times 0.5\ H_2O$) C, H, N.

#### 4.3.37. 4-(*N*-(2-(3-(*tert*-Butoxycarbonyl)phenylamino)-2-oxoethyl)sulfamoyl)benzamidinium trifluoroacetate (37)

Yield 32%, mp 212–215 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.53 (s, 9H), 3.78 (s, 2H), 7.40 (dd, $J$ = 7.8 Hz, $J$ = 7.8 Hz, 1H), 7.56–7.58 (m, 1H), 7.71–7.73 (m, 1H), 7.96, 8.03 (each d, $J$ = 8.5 Hz, 4H), 8.04 (dd, $J$ = 1.9 Hz, $J$ = 1.9 Hz, 1H), 8.41 (s, 1H), 9.33 (s, 4H), 10.18 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 27.9, 46.2, 80.9, 119.8, 123.5, 124.1, 126.9, 128.5, 129.2, 132.0, 133.7, 138.9, 144.8, 164.8, 165.0, 166.9. LC–MS (ESI) purity 99%, $m/z$ 433.4 $[M+H]^+$, (method c). Anal ($C_{22}H_{25}F_3N_4O_7S \times 1.3\ H_2O$) C, H, N.

#### 4.3.38. 4-(*N*-(3-(4-(*tert*-Butoxycarbonyl)phenylamino)-3-oxopropyl)sulfamoyl)benzamidinium trifluoroacetate (38)

Yield 19%, mp 223–225 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.52 (s, 9H), 2.57 (t, $J$ = 7.0 Hz, 2H), 3.07 (t, $J$ = 7.0 Hz, 2H), 7.66 (d, $J$ = 8.9 Hz 2H), 7.82 (d, $J$ = 8.8 Hz, 2H), 7.97, 8.00 (each d, $J$ = 8.7 Hz, 4H), 9.38 (s, 4H), 10.28 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 28.0, 36.8, 38.8, 80.4, 118.4, 125.8, 127.0, 129.4, 130.2, 132.2, 143.1, 144.7, 158.4 (q, $J$ = 31.3 Hz), 164.7, 165.1, 169.3. LC–MS (ESI) purity 99%, $m/z$ 447.3 $[M+H]^+$, (method c). Anal ($C_{23}H_{27}F_3N_4O_7S$) C, H, N.

#### 4.3.39. 4-(*N*-(3-(3-(*tert*-Butoxycarbonyl)phenylamino)-3-oxopropyl)sulfamoyl)benzamidinium trifluoroacetate (39)

Yield 51%, mp 194–199 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.53 (s, 9H), 2.54 (t, $J$ = 7.0 Hz, 2H), 3.05–3.09 (m, 2H), 7.39 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.55–7.57 (m, 1H), 7.78–7.80 (m, 1H), 7.97–8.05 (m, 5H), 8.13 (dd, $J$ = 1.9 Hz, $J$ = 1.9 Hz, 1H), 9.30, 9.44 (each s, 4H), 10.16 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 27.9, 36.7, 39.1, 80.9, 119.7, 123.4, 123.8, 126.9, 128.7, 129.0, 132.2, 134.6, 139.4, 143.8, 164.9, 164.9, 169.1. LC–MS (ESI) purity 96%, $m/z$ 447.3 $[M+H]^+$, (method c). Anal ($C_{23}H_{27}F_3N_4O_7S \times 1.0\ H_2O$) C, H, N.

#### 4.3.40. 4-(*N*-(4-(4-(*tert*-Butoxycarbonyl)phenylamino)-4-oxobutyl)sulfamoyl)benzamidinium trifluoroacetate (40)

Yield 14%, mp 122–137 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.52 (s, 9H), 1.71 (tt, $J$ = 7.3 Hz, $J$ = 7.3 Hz, 2H), 2.38 (t, $J$ = 7.3 Hz, 2H), 2.81–2.85 (m, 2H), 7.66 (d, $J$ = 8.9 Hz, 2H), 7.82 (d, $J$ = 8.9 Hz, 2H), 7.93–7.99 (m, 5H), 9.22, 9.43 (each s, 4H), 10.18 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 25.0, 28.0, 33.4, 42.3, 80.4, 118.4, 125.6, 126.8, 128.7, 130.2, 132.1, 143.2, 144.1, 164.7, 165.0, 171.2. LC–MS (ESI) purity 95%, $m/z$ 461.3 $[M+H]^+$, (method c). Anal ($C_{24}H_{29}F_3N_4O_7S \times 1.1\ H_2O$) C, H, N.

#### 4.3.41. 4-(*N*-(4-(3-(*tert*-Butoxycarbonyl)phenylamino)-4-oxobutyl)sulfamoyl)benzamidinium trifluoroacetate (41)

Yield 70%, mp 123–138 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.53 (s, 9H), 1.72 (tt, $J$ = 7.3 Hz, $J$ = 7.3 Hz, 2H), 2.36 (t, $J$ = 7.3 Hz, 2H), 2.82–2.85 (m, 2H), 7.39 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.54–7.56 (m, 1H), 7.79–7.82 (m, 1H), 7.94–7.98 (m, 5H), 8.13 (dd, $J$ = 1.7 Hz, $J$ = 1.7 Hz, 1H), 9.44, 9.45 (each s, 4H), 10.08 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 25.1, 27.9, 33.3, 42.3, 80.9, 119.6, 123.3, 123.7, 126.9, 129.0, 129.4, 132.0, 132.1, 139.5, 145.1, 158.7 (q, $J$ = 32.8), 165.0, 165.2, 170.9. LC–MS (ESI) purity 99%, $m/z$ 461.4 $[M+H]^+$, (method c). Anal ($C_{24}H_{29}F_3N_4O_7S \times 1.5\ H_2O$) C, H, N.

#### 4.3.42. 3-(*N*-(2-(4-(*tert*-Butoxycarbonyl)phenylamino)-2-oxoethyl)sulfamoyl)benzamidinium trifluoroacetate (42)

Yield 39%, mp 112–125 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.52 (s, 9H), 3.79 (d, $J$ = 6.0 Hz, 2H), 7.59 (d, $J$ = 8.8 Hz, 2H), 7.83 (d, $J$ = 8.98 Hz, 2H), 7.83 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 8.01–8.03 (m, 1H), 8.14–8.16 (m, 1H), 8.21 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 8.32 (t, $J$ = 6.0 Hz, 1H), 9.34, 9.48 (each s, 4H), 10.28 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 28.0, 45.9, 80.5, 118.6, 126.2, 126.4, 129.3, 130.2, 130.2, 131.5, 132.2, 141.6, 142.6, 158.6 (q, $J$ = 32.5 Hz), 164.7, 164.9, 166.9. LC–MS (ESI) purity 96%, $m/z$ 433.4 $[M+H]^+$, (method c). Anal ($C_{22}H_{25}F_3N_4O_7S \times 1.9\ H_2O$) C, H, N.

#### 4.3.43. 3-(*N*-(2-(3-(*tert*-Butoxycarbonyl)phenylamino)-2-oxoethyl)sulfamoyl)benzamidinium trifluoroacetate (43)

Yield 18%, mp 168–173 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.53 (s, 9H), 3.77 (s, 2H), 7.40 (dd, $J$ = 7.8 Hz, $J$ = 7.8 Hz, 1H), 7.56–7.59 (m, 1H), 7.72–7.75 (m, 1H), 7.83 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 8.01–8.03 (m, 1H), 8.05 (dd, $J$ = 1.9 Hz, $J$ = 1.9 Hz, 1H), 8.15–8.17 (m, 1H), 8.21 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 9.42 (s, 4H), 10.18 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 27.9, 45.9, 81.0, 119.9, 123.5, 124.2, 126.4, 129.2, 129.3, 130.2, 131.5, 132.2, 132.0, 138.9, 141.6, 158.6 (q, $J$ = 31.0 Hz), 164.8, 165.0, 166.8. LC–MS (ESI) purity 99%, $m/z$ 433.3 $[M+H]^+$, (method c). Anal ($C_{22}H_{25}F_3N_4O_7S$) C, H, N.

#### 4.3.44. 3-(*N*-(3-(4-(*tert*-Butoxycarbonyl)phenylamino)-3-oxopropyl)sulfamoyl)benzamidinium trifluoroacetate (44)

Yield 70%, mp 127–135 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.52 (s, 9H), 2.56 (t, $J$ = 7.0 Hz, 2H), 3.07–3.11 (m, 2H), 7.66 (d, $J$ = 8.8 Hz, 2H), 7.83 (d, $J$ = 8.8 Hz, 2H), 7.84 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.97 (t, $J$ = 6.0 Hz, 1H), 8.02–8.04 (m, 1H), 8.11–8.13 (m, 1H), 8.18 (dd, $J$ = 1.7 Hz, $J$ = 1.7 Hz, 1H), 9.40, 9.50 (each s, 4H), 10.28 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 28.0, 36.8, 38.8, 80.4, 118.5, 125.8, 126.4, 129.6, 130.2, 130.3, 131.3, 132.2, 141.3, 143.2, 158.6 (q, $J$ = 31.9 Hz), 164.7, 165.1, 169.4. LC–MS (ESI) purity 99%, $m/z$ 447.3 $[M+H]^+$, (method c). Anal ($C_{23}H_{27}F_3N_4O_7S \times 1.8\ H_2O$) C, H, N.

#### 4.3.45. 3-(*N*-(3-(3-(*tert*-Butoxycarbonyl)phenylamino)-3-oxopropyl)sulfamoyl)benzamidinium trifluoroacetate (45)

Yield 36%, mp 100–110 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.53 (s, 9H), 2.54 (t, $J$ = 6.9 Hz, 2H), 3.07–3.11 (m, 2H), 7.40 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.55–7.57 (m, 1H), 7.79–7.81 (m, 1H), 7.85 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.96 (t, $J$ = 5.7 Hz, 1H), 8.01–8.03 (m, 1H), 8.11–8.13 (m, 2H), 8.17 (dd, $J$ = 1.7 Hz, $J$ = 1.7 Hz, 1H), 9.19, 9.49 (each s, 4H), 10.15 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 27.9, 36.8, 39.0, 80.9, 119.7, 123.4, 123.8, 125.7, 129.0, 129.9, 130.1, 131.3, 192.6, 132.3, 139.4, 141.1, 164.6, 164.9, 169.2. LC–MS (ESI) purity 99%, $m/z$ 447.3 $[M+H]^+$, (method c). Anal ($C_{23}H_{27}F_3N_4O_7S \times 1.9\ H_2O$) C, H, N.

#### 4.3.46. 3-(*N*-(4-(4-(*tert*-Butoxycarbonyl)phenylamino)-4-oxobutyl)sulfamoyl)benzamidinium trifluoroacetate (46)

Yield 32%, mp 100–111 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.52 (s, 9H), 1.70 (tt, $J$ = 7.3 Hz, $J$ = 7.3 Hz, 2H), 2.37 (t, $J$ = 7.3 Hz, 2H), 2.82–3.86 (m, 2H), 7.66 (d, $J$ = 8.9 Hz, 2H), 7.80–7.87 (m, 4H), 8.00–8.02 (m, 1H), 8.09–8.11 (m, 1H), 8.16 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 9.32, 9.49 (each s, 4H), 10.19 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 24.9, 28.0, 33.3, 42.2, 80.4, 118.4, 125.6, 126.3, 129.5, 130.2, 130.3, 131.2, 132.1, 141.5, 143.3, 158.5 (q, $J$ = 32.8 Hz),

164.7, 165.0, 171.2. LC–MS (ESI) purity 98%, *m/z* 461.4 $[M+H]^+$, (method c). Anal ($C_{24}H_{29}F_3N_4O_7S \times 1.6\ H_2O$) C, H, N.

#### 4.3.47. 3-(*N*-(4-(3-(*tert*-Butoxycarbonyl)phenylamino)-4-oxobutyl)sulfamoyl)benzamidinium trifluoroacetate (47)

Yield 47%, mp 91–100 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.53 (s, 9H), 1.71 (tt, $J$ = 7.3 Hz, $J$ = 7.3 Hz, 2H), 2.35 (t, $J$ = 7.3 Hz, 2H), 2.82–2.86 (m, 2H), 7.39 (dd, $J$ = 8.1 Hz, $J$ = 8.1 Hz, 1H), 7.54–7.56 (m, 1H), 7.80–7.82 (m, 1H), 7.84 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.87 (t, $J$ = 6.0 Hz, 1H), 8.01–8.03 (m, 1H), 8.10–8.12 (m, 2H), 8.16 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 9.39, 9.49 (each s, 4H), 10.08 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 25.1, 27.9, 33.3, 42.2, 80.9, 119.6, 123.3, 123.7, 126.3, 129.0, 129.6, 130.3 131.2, 132.1, 131.9, 139.6, 141.5, 158.6 (q, $J$ = 32.8 Hz), 165.0, 165.1, 171.0. LC–MS (ESI) purity 99%, *m/z* 461.3 $[M+H]^+$, (method c). Anal ($C_{24}H_{29}F_3N_4O_7S \times 1.7\ H_2O$) C, H, N.

#### 4.3.48. 4-(*N*-(2-(4-Carboxylphenylamino)-2-oxoethyl)-sulfamoyl)benzamidinium trifluoroacetate (48)

Yield >99%, mp >250 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.81 (d, $J$ = 6.0 Hz, 2H), 7.58 (d, $J$ = 8.8 Hz, 2H), 7.86 (d, $J$ = 8.9 Hz, 2H), 7.96, 8.02 (each d, $J$ = 8.7 Hz, 4H), 8.43 (t, $J$ = 6.1 Hz, 1H), 9.35, 9.44 (each s, 4H), 10.29 (s, 1H), 12.66 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 45.9, 118.7, 125.6, 127.0, 129.2, 130.5, 131.9, 142.6, 145.4, 165.0, 166.9, 167.0. LC–MS (ESI) purity 98%, *m/z* 377.5 $[M+H]^+$, (method d). Anal ($C_{18}H_{17}F_3N_4O_7S \times 1.1\ H_2O$) C, H, N.

#### 4.3.49. 4-(*N*-(2-(3-Carboxylphenylamino)-2-oxoethyl)-sulfamoyl)benzamidinium trifluoroacetate (49)

Yield >99%, mp 236–240 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.78 (d, $J$ = 6.0 Hz, 2H), 7.40 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.60–7.62 (m, 1H), 7.69–7.72 (m, 1H), 7.97, 8.03 (each d, $J$ = 8.8 Hz, 4H), 8.12 (dd, $J$ = 1.9 Hz, $J$ = 1.9 Hz, 1H), 8.42 (t, $J$ = 6.0 Hz, 1H), 9.36, 9.45 (each s, 4H), 10.18 (s, 1H), 12.85 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 45.9, 120.2, 123.5, 124.5, 127.0, 129.1, 129.2, 131.5, 131.9, 138.9, 145.4, 158.7 (q, $J$ = 32.8 Hz), 165.0, 166.6, 167.2. LC–MS (ESI) purity 94%, *m/z* 377.4 $[M+H]^+$, (method d). Anal ($C_{18}H_{17}F_3N_4O_7S \times 1.4\ H_2O$) C, H, N.

#### 4.3.50. 4-(*N*-(3-(4-Carboxylphenylamino)-3-oxopropyl)-sulfamoyl)benzamidinium trifluoroacetate (50)

Yield >99%, mp 146–152 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 2.57 (t, $J$ = 7.0 Hz, 2H), 3.07–3.10 (m, 2H), 7.66 (d, $J$ = 8.9 Hz, 2H), 7.86 (d, $J$ = 8.9 Hz, 2H), 7.97, 8.00 (each d, $J$ = 8.7 Hz, 4H), 8.05 (t, $J$ = 5.7 Hz, 1H), 9.37, 9.45 (each s, 4H), 10.27 (s, 1H), 12.63 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 36.8, 38.9, 118.5, 125.2, 127.0, 129.4, 130.5, 132.1, 143.1, 144.7, 158.5 (q, $J$ = 31.9 Hz), 165.1, 167.0, 169.3. LC–MS (ESI) purity 99%, *m/z* 391.3 $[M+H]^+$, (method d). Anal ($C_{19}H_{19}F_3N_4O_7S \times 1.8\ H_2O$) C, H, N.

#### 4.3.51. 4-(*N*-(3-(3-Carboxylphenylamino)-3-oxopropyl)-sulfamoyl)benzamidinium trifluoroacetate (51)

Yield >99%, mp 122–132 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 2.55 (t, $J$ = 7.0 Hz, 2H), 3.06–3.10 (m, 2H), 7.40 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.59–7.61 (m, 1H), 7.75–7.77 (m, 1H), 7.99, 8.00 (each s, 4H), 8.04 (t, $J$ = 5.7 Hz, 1H), 8.21 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 9.44, 9.45 (each s, 4H), 10.16 (s, 1H), 12.81 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 36.8, 39.0, 120.1, 123.3, 124.1, 127.0, 129.1, 129.4, 131.4, 132.2, 139.4, 144.8, 158.7 (q, $J$ = 31.0 Hz), 165.2, 167.3, 169.0. LC–MS (ESI) purity 97%, *m/z* 391.5 $[M+H]^+$, (method d). Anal ($C_{19}H_{19}F_3N_4O_7S \times 1.0\ H_2O$) C, H, N.

#### 4.3.52. 4-(*N*-(4-(4-Carboxylphenylamino)-4-oxobutyl)-sulfamoyl)benzamidinium trifluoroacetate (52)

Yield >99%, mp 156–162 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.72 (tt, $J$ = 7.0 Hz, $J$ = 7.0 Hz, 2H), 2.38 (t, $J$ = 7.3 Hz, 2H), 2.82–2.86 (m, 2H), 7.66 (d, $J$ = 8.8 Hz, 2H), 7.86 (d, $J$ = 8.8 Hz, 2H), 7.95 (t, $J$ = 5.7 Hz, 1H), 7.98 (s, 4H), 9.45 (s, 4H), 10.19 (s, 1H), 12.64 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 25.1, 33.4, 42.3, 118.4, 125.1, 126.9, 129.5, 130.5, 132.1, 143.4, 145.1, 165.2, 167.0, 171.2. LC–MS (ESI) purity 95%, *m/z* 405.4 $[M+H]^+$, (method d). Anal ($C_{20}H_{21}F_3N_4O_7S \times 1.1\ H_2O$) C, H, N.

#### 4.3.53. 4-(*N*-(4-(3-Carboxylphenylamino)-4-oxobutyl)-sulfamoyl)benzamidinium trifluoroacetate (53)

Yield >99%, mp 167–171 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.72 (tt, $J$ = 7.3 Hz, $J$ = 7.3 Hz, 2H), 2.36 (t, $J$ = 7.3 Hz, 2H), 2.82–2.86 (m, 2H), 7.39 (dd, $J$ = 8.2 Hz, $J$ = 8.2 Hz, 1H), 7.58–7.60 (m, 1H), 7.75–7.78 (m, 1H), 7.95 (t, $J$ = 6.0 Hz, 1H), 7.97, 7.98 (each s, 4H), 8.20 (dd, $J$ = 1.9 Hz, $J$ = 1.9 Hz, 1H), 9.37, 9.44 (each s, 4H), 10.06 (s, 1H), 12.90 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 25.1, 33.3, 42.3, 119.9, 123.3, 124.0, 126.9, 129.0, 129.5, 131.4, 132.1, 139.5, 145.1, 158.6 (q, $J$ = 31.0 Hz), 165.1, 167.3, 170.9. LC–MS (ESI) purity 96%, *m/z* 405.4 $[M+H]^+$, (method d). Anal ($C_{20}H_{21}F_3N_4O_7S \times 1.0\ H_2O$) C, H, N.

#### 4.3.54. 3-(*N*-(2-(4-Carboxylphenylamino)-2-oxoethyl)-sulfamoyl)benzamidinium trifluoroacetate (54)

Yield >99%, mp 140–151 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.80 (d, $J$ = 6.0 Hz, 2H), 7.59 (d, $J$ = 8.8 Hz, 2H), 7.84 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.87 (d, $J$ = 8.9 Hz, 2H), 8.01–8.03 (m, 1H), 8.14–8.16 (m, 1H), 8.21 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 8.24 (t, $J$ = 6.1 Hz, 1H), 9.39, 9.48 (each s, 4H), 10.28 (s, 1H), 12.72 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 46.0, 118.6, 125.6, 126.3, 129.3, 130.2, 131.5, 132.2, 130.5, 141.6, 142.6, 158.7 (q, $J$ = 31.9 Hz, 165.0, 166.9, 167.0. LC–MS (ESI) purity 95%, *m/z* 377.4 $[M+H]^+$, (method d). Anal ($C_{18}H_{17}F_3N_4O_7S \times 2.0\ H_2O$) C, H, N.

#### 4.3.55. 3-(*N*-(2-(3-Carboxylphenylamino)-2-oxoethyl)-sulfamoyl)benzamidinium trifluoroacetate (55)

Yield >99%, mp >250 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.77 (d, $J$ = 6.0 Hz, 2H), 7.41 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.61–7.63 (m, 1H), 7.70–7.73 (m, 1H), 7.83 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 8.01–8.03 (m, 1H), 8.11 (dd, $J$ = 1.9 Hz, $J$ = 1.9 Hz, 1H), 8.15–8.17 (m, 1H), 8.21 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 2H), 8.31 (t, $J$ = 6.2 Hz, 1H), 9.37, 9.49 (each s, 4H), 10.16 (s, 1H), 12.96 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 45.9, 120.2, 123.5, 124.4, 126.3, 129.2, 129.3, 130.2, 131.5, 132.2, 131.5, 138.8, 141.6, 158.7 (q, $J$ = 31.0 Hz), 165.0, 166.7, 167.2. LC–MS (ESI) purity 97%, *m/z* 377.3 $[M+H]^+$, (method d). Anal ($C_{18}H_{17}F_3N_4O_7S \times 0.5\ H_2O$) C, H, N.

#### 4.3.56. 3-(*N*-(3-(4-Carboxylphenylamino)-3-oxopropyl)-sulfamoyl)benzamidinium trifluoroacetate (56)

Yield >99%, mp 224–229 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 2.56 (t, $J$ = 6.9 Hz, 2H), 3.07–3.11 (m, 2H), 7.66 (d, $J$ = 8.9 Hz, 2H), 7.84 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.86 (d, $J$ = 8.8 Hz, 2H), 7.97 (t, $J$ = 6.0 Hz, 1H), 8.01–8.03 (m, 1H), 8.11–8.13 (m, 1H), 8.18 (dd, $J$ = 1.9 Hz, $J$ = 1.9 Hz), 9.43, 9.49 (each s, 4H), 10.27 (s, 1H), 12.66 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 36.9, 38.8, 118.5, 125.3, 126.4, 129.6, 130.3, 131.3, 132.2, 130.5, 141.3, 143.2, 158.8 (q, $J$ = 31.0 Hz), 165.1, 167.0, 169.4. LC–MS (ESI) purity 99%, *m/z* 391.4 $[M+H]^+$, (method d). Anal ($C_{19}H_{19}F_3N_4O_7S \times 1.1\ H_2O$) C, H, N.

#### 4.3.57. 3-(*N*-(3-(3-Carboxylphenylamino)-3-oxopropyl)-sulfamoyl)benzamidinium trifluoroacetate (57)

Yield >99%, mp 113–125 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 2.53 (t, $J$ = 6.9 Hz, 2H), 3.07–3.11 (m, 2H), 7.40 (dd, $J$ = 8.2 Hz, $J$ = 8.2 Hz, 1H), 7.59–7.61 (m, 1H), 7.76–7.78 (m, 1H), 7.85 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.96 (t, $J$ = 6.0 Hz, 1H), 8.01–8.03 (m, 1H), 8.11–8.13 (m, 1H), 8.16–8.20 (m, 2H), 9.35, 9.49 (each s, 4H), 10.15 (s, 1H), 12.89 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 36.8, 38.9, 120.1, 123.3, 124.1, 126.4, 129.1, 129.6, 130.3, 131.3, 132.2, 131.4, 139.4, 141.3, 158.5 (q, $J$ = 31.3 Hz), 165.1, 167.3, 169.1. LC–MS

(ESI) purity 98%, *m/z* 391.3 $[M+H]^+$, (method d). Anal ($C_{19}H_{19}F_3N_4O_7S \times 1.6\ H_2O$) C, H, N.

#### 4.3.58. 3-(*N*-(4-(4-Carboxylphenylamino)-4-oxobutyl)-sulfamoyl)benzamidinium trifluoroacetate (58)

Yield >99%, mp 152–155 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.71 (tt, $J$ = 7.0 Hz, $J$ = 7.0 Hz, 2H), 2.38 (t, $J$ = 7.3 Hz, 2H), 2.83–2.86 (m, 2H), 7.66 (d, $J$ = 8.8 Hz, 2H), 7.82–7.87 (m, 4H), 8.00–8.02 (m, 1H), 8.09–8.11 (m, 1H), 8.16 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 9.36, 9.49 (each s, 4H), 10.19 (s, 1H), 12.66 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 25.0, 33.4, 42.2, 118.4, 125.1, 126.3, 129.5, 130.3, 131.2, 132.1, 130.5, 141.5, 143.3, 158.7 (q, $J$ = 31.9 Hz), 165.1, 167.0, 171.2. LC–MS (ESI) purity 95%, *m/z* 405.5 $[M+H]^+$, (method d). Anal ($C_{20}H_{21}F_3N_4O_7S \times 0.6\ H_2O$) C, H, N.

#### 4.3.59. 3-(*N*-(4-(3-Carboxylphenylamino)-4-oxobutyl)-sulfamoyl)benzamidinium trifluoroacetate (59)

Yield >99%, mp 92–111 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.71 (tt, $J$ = 7.2 Hz, $J$ = 7.2 Hz, 2H), 2.35 (t, $J$ = 7.3 Hz, $J$ = 7.3 Hz, 2H), 2.82–2.86 (m, 2H), 7.39 (dd, $J$ = 8.2 Hz, $J$ = 8.2 Hz, 1H), 7.58–7.60 (m, 1H), 7.76–7.78 (m, 1H), 7.84 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.87 (t, $J$ = 6.0 Hz, 1H), 8.00–8.02 (m, 1H), 8.10–8.12 (m, 1H), 8.16 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 8.20 (dd, $J$ = 1.9 Hz, $J$ = 1.9 Hz, 1H), 9.35, 9.49 (each s, 4H), 10.06 (s, 1H), 12.81 (s, 1H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 25.1, 32.2, 42.2, 119.9, 123.2, 123.9, 126.3, 129.0, 129.5, 130.3, 131.1, 132.1, 131.4, 139.1, 141.5, 158.5 (q, $J$ = 33.7 Hz, 165.0, 167.2, 170.9. LC–MS (ESI) purity 96%, *m/z* 405.4 $[M+H]^+$, (method d). Anal ($C_{20}H_{21}F_3N_4O_7S \times 1.0\ H_2O$) C, H, N.

#### 4.3.60. 4-(*N*-(2-(4-Amidiniobenzylamino)-2-oxoethyl)-sulfamoyl)benzamidinium bis(trifluoroacetate) (60)

Yield 26%, mp 211–216 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.55 (d, $J$ = 6.0 Hz, 2H), 4.34 (d, $J$ = 6.0 Hz, 2H), 7.45 (d, $J$ = 8.2 Hz, 2H), 7.76 (d, $J$ = 8.5 Hz, 2H), 7.98, 8.01 (each d, $J$ = 8.5 Hz, 4H), 8.40 (t, $J$ = 6.0 Hz, 1H), 8.61 (t, $J$ = 6.2 Hz, 1H), 9.22, 9.25, 9.47, 9.49 (each s, 8H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 41.9, 45.4, 126.7, 127.0, 127.6, 128.2, 129.3, 132.1, 144.9, 145.7, 158.7 (q, $J$ = 31.0 Hz), 165.1, 165.6, 168.0. LC–MS (ESI) purity 99%, *m/z* 389.4 $[M+H]^+$, (method d). Anal ($C_{21}H_{22}F_6N_6O_7S \times 0.7\ H_2O$) C, H, N.

#### 4.3.61. 4-(*N*-(3-(4-Amidiniobenzylamino)-3-oxopropyl)-sulfamoyl)benzamidinium bis(trifluoroacetate) (61)

Yield 28%, mp 210–211 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 2.39 (t, $J$ = 7.3 Hz, 2H), 2.98–3.02 (m, 2H), 4.33 (d, $J$ = 6.0 Hz, 2H), 7.45 (d, $J$ = 8.5 Hz, 2H), 7.76 (d, $J$ = 8.2 Hz, 2H), 7.99 (s, 5H), 8.56 (t, $J$ = 6.0 Hz, 1H), 9.26, 9.47, 9.53 (each s, 8H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 35.7, 39.3, 41.9, 126.7, 127.0, 127.6, 128.2, 129.4, 132.2, 144.7, 146.0, 158.9 (q, $J$ = 31.9 Hz), 165.2, 165.6, 170.0. LC–MS (ESI) purity 99%, *m/z* 403.5 $[M+H]^+$, (method d). Anal ($C_{22}H_{24}F_6N_6O_7S \times 0.3\ H_2O$) C, H, N.

#### 4.3.62. 4-(*N*-(4-(4-Amidiniobenzylamino)-4-oxobutyl)-sulfamoyl)benzamidinium bis(trifluoroacetate) (62)

Yield 10%, mp 238–239 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.65 (tt, $J$ = 7.3 Hz, $J$ = 7.3 Hz, 2H), 2.19 (t, $J$ = 7.3 Hz, 2H), 2.78–2.81 (m, 2H), 4.32 (d, $J$ = 5.7 Hz, 2H), 7.44 (d, $J$ = 8.5 Hz, 2H), 7.76 (d, $J$ = 8.2 Hz, 2H), 7.93 (t, $J$ = 5.7 Hz, 1H), 7.98 (s, 4H), 8.46 (t, $J$ = 6.0 Hz, 1H), 9.25, 9.47, 9.53 (each s, 8H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 25.5, 32.3, 41.9, 42.4, 126.6, 126.9, 127.5, 128.2, 129.4, 132.1, 145.1, 146.2, 158.8 (q, $J$ = 31.9 Hz), 165.2, 165.6, 171.8. LC–MS (ESI) purity 98%, *m/z* 417.4 $[M+H]^+$, (method d). Anal ($C_{23}H_{26}F_6N_6O_7S \times 0.4\ H_2O$) C, H, N.

#### 4.3.63. 3-(*N*-(2-(4-Amidiniobenzylamino)-2-oxoethyl)-sulfamoyl)benzamidinium (bis)trifluoroacetate (63)

Yield 49%, mp 232–235 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.56 (d, $J$ = 6.3 Hz, 2H), 4.33 (d, $J$ = 6.0 Hz, 2H), 7.44 (d, $J$ = 8.5 Hz, 2H), 7.76 (d, $J$ = 8.2 Hz, 2H), 7.84 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 8.03–8.05 (m, 1H), 8.12–8.14 (m, 1H), 8.19 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 8.31 (t, $J$ = 6.3 Hz, 1H), 8.59 (t, $J$ = 6.0 Hz, 1H), 9.26, 9.50 (each s, 8H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 41.9, 45.3, 117.2 (q, $J$ = 297.0 Hz), 126.4, 126.7, 127.5, 128.2, 129.4, 130.3, 131.4, 132.2, 141.2, 145.7, 158.8 (q, $J$ = 31.0 Hz), 165.1, 165.6, 168.1. LC–MS (ESI) purity 97%, *m/z* 389.5 $[M+H]^+$, (method d). Anal ($C_{21}H_{22}F_6N_6O_7S \times 0.5\ H_2O$) C, H, N.

#### 4.3.64. 3-(*N*-(3-(4-Amidiniobenzylamino)-3-oxopropyl)-sulfamoyl)benzamidinium bis(trifluoroacetate) (64)

Yield 71%, mp 216–220 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 2.38 (t, $J$ = 7.3 Hz, 2H), 3.00–3.04 (m, 2H), 4.33 (d, $J$ = 6.0 Hz, 2H), 7.45 (d, $J$ = 8.5 Hz, 2H), 7.75 (d, $J$ = 8.2 Hz, 2H), 7.84 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.91 (t, $J$ = 5.7 Hz, 1H), 8.02–8.04 (m, 1H), 8.10–8.12 (m, 1H), 8.17 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 8.56 (t, $J$ = 6.0 Hz, 1H), 9.24, 9.26, 9.48, 9.51 (each s, 8H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 35.6, 41.9, 126.3, 126.6, 127.6, 128.2, 129.6, 130.3, 131.2, 132.1, 141.3, 146.0, 158.8 (q, $J$ = 31.0 Hz), 165.1, 165.6, 170.1. LC–MS (ESI) purity 99%, *m/z* 403.5 $[M+H]^+$, (method d). Anal ($C_{22}H_{24}F_6N_6O_7S \times 1.0\ H_2O$) C, H, N.

#### 4.3.65. 3-(*N*-(4-(4-Amidiniobenzylamino)-4-oxobutyl)-sulfamoyl)benzamidinium bis(trifluoroacetate) (65)

Yield 39%, mp 212–218 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.63 (tt, $J$ = 7.3 Hz, $J$ = 7.3 Hz, 2H), 2.18 (t, $J$ = 7.4 Hz, 2H), 2.78–2.82 (m, 2H), 4.32 (d, $J$ = 6.0 Hz, 2H), 7.43 (d, $J$ = 8.5 Hz, 2H), 7.76 (d, $J$ = 8.5 Hz, 2H), 7.82–7.86 (m, 2H), 8.02–8.04 (m, 1H), 8.08–8.10 (m, 1H), 8.16 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 8.47 (t, $J$ = 6.0 Hz, 1H), 9.24, 9.26, 9.49, 9.51 (each s, 8H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 25.4, 32.3, 41.9, 42.4, 117.3 (q, $J$ = 297.9 Hz), 126.3, 126.6, 127.5, 128.3, 129.6, 130.3, 131.2, 132.1, 141.5, 146.2, 158.8 (q, $J$ = 31.0 Hz), 165.2, 165.6, 171.9. LC–MS (ESI) purity 98%, *m/z* 417.3 $[M+H]^+$, (method d). Anal ($C_{23}H_{26}F_6N_6O_7S \times 2.0\ H_2O$) C, H, N.

#### 4.3.66. (*S*)-4-(*N*-(1-(4-Amidiniobenzylamino)-1-oxo-3-phenylpropan-2-yl)sulfamoyl)benzamidinium bis(trifluoroacetate) (66)

Yield 10%, mp 256–259 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 2.66–2.72, 2.86–2.89 (each m, 2H), 4.08–4.14 (m, 2H), 4.24 (dd, $J$ = 16.1 Hz, $J$ = 6.6 Hz, 1H), 7.09–7.11 (m, 2H), 7.17–7.18 (m, 3H), 7.20 (d, $J$ = 8.2 Hz, 2H), 7.69 (d, $J$ = 8.2 Hz, 2H), 7.75, 7.84 (each d, $J$ = 8.8 Hz, 4H), 8.56 (d, $J$ = 8.9 Hz, 1H), 8.63 (t, $J$ = 5.7 Hz, 1H), 9.05, 9.23, 9.32, 9.43 (each s, 8H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 38.5, 41.7, 58.2, 117.4 (q, $J$ = 297.9 Hz), 126.6, 126.7, 126.7, 127.3, 128.2, 128.4, 128.9, 129.4, 131.5, 136.8, 145.5, 145.9, 158.7 (q, $J$ = 31.0 Hz), 164.9, 165.5, 170.4. LC–MS (ESI) purity 99%, *m/z* 479.3 $[M+H]^+$, (method d). Anal ($C_{28}H_{28}F_6N_6O_7S \times 2.9\ H_2O$) C, H, N.

#### 4.3.67. (*R*)-4-(*N*-(1-(4-Amidiniobenzylamino)-1-oxo-3-phenylpropan-2-yl)sulfamoyl)benzamidinium bis(trifluoroacetate) (67)

Yield 22%, mp 255–257 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 2.68, 2.84 (each dd, $J$ = 13.6 Hz, $J$ = 6.6 Hz, 2H), 4.06–4.13 (m, 2H), 4.24 (dd, $J$ = 16.1 Hz, $J$ = 6.3 Hz, 1H), 7.09–7.11 (m, 2H), 7.16–7.18 (m, 3H), 7.20 (d, $J$ = 8.5 Hz, 2H), 7.69 (d, $J$ = 8.6 Hz, 2H), 7.75, 7.84 (each d, $J$ = 8.8 Hz, 4H), 8.56 (d, $J$ = 8.8 Hz, 1H), 8.64 (t, $J$ = 6.0 Hz, 1H), 9.02, 9.23, 9.29, 9.42 (each s, 8H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 38.4, 41.7, 58.2, 126.5, 126.7, 126.7, 127.3, 128.1, 128.4, 128.9, 129.4, 131.5, 136.8, 145.5, 145.9, 158.7 (q, $J$ = 31.9 Hz), 164.9, 165.5, 170.4. LC–MS (ESI) purity 99%, *m/z* 479.3 $[M+H]^+$, (method d). Anal ($C_{28}H_{28}F_6N_6O_7S \times 2.2\ H_2O$) C, H, N.

#### 4.3.68. (*S*)-4-(*N*-(1-(4-Amidiniobenzylamino)-3-benzyloxy-1-oxopropan-2-yl)sulfamoyl)benzamidinium bis(trifluoroacetate) (68)

Yield 14%, mp 243–244 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.46, 3.50 (each dd, $J$ = 9.8 Hz, 6.0 Hz, 2H), 4.10 (dd, $J$ = 8.2 Hz, $J$ = 6.3 Hz, 1H), 4.23, 4.33 (each dd, $J$ = 15.1 Hz, $J$ = 6.0 Hz, 2H), 4.33–4.35 (m, 2H), 7.19–7.21 (m, 2H), 7.25–7.32 (m, 3H), 7.40 (d, $J$ = 8.5 Hz, 2H), 7.69 (d, $J$ = 8.2 Hz, 2H), 7.92, 8.00 (each d, $J$ = 8.5 Hz. 4H), 8.55 (d, $J$ = 8.2 Hz, 1H), 8.73 (t, $J$ = 6.0 Hz, 1H), 9.21, 9.23, 9.46, 9.50 (each s, 8H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 41.9, 56.4, 69.9, 72.2, 117.3 (q, $J$ = 197.9 Hz), 126.6, 127.0, 127.3, 127.6, 128.1, 128.3, 129.0, 131.7, 137.9, 145.5, 145.8, 158.7 (q, $J$ = 31.0 Hz), 164.9, 165.6, 169.0. LC–MS (ESI) purity 99%, *m/z* 509.3 $[M+H]^+$, (method d). Anal ($C_{29}H_{30}F_6N_6O_8S \times 2.5\ H_2O$) C, H, N.

#### 4.3.69. (*R*)-4-(*N*-(1-(4-Amidiniobenzylamino)-3-benzyloxy-1-oxopropan-2-yl)sulfamoyl)benzamidinium bis(trifluoroacetate) (69)

Yield 9%, mp 245–249 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.46, 3.48 (each dd, $J$ = 10.1 Hz, 6.3 Hz, 2H), 4.10 (dd, 8.2 Hz, $J$ = 6.3 Hz, 1H), 4.23, 4.32 (each dd, $J$ = 16.1 Hz, $J$ = 5.7 Hz, 2H), 4.33–4.35 (m, 2H), 7.19–7.21 (m, 2H), 7.26–7.32 (m, 3H), 7.40 (d, $J$ = 8.5 Hz, 2H), 7.69 (d, $J$ = 8.5 Hz, 2H), 7.92, 7.99 (each d, $J$ = 8.5 Hz. 4H), 8.55 (d, $J$ = 8.2 Hz, 1H), 8.72 (t, $J$ = 6.0 Hz, 1H), 9.20, 9.23, 9.46, 9.49 (each s, 8H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 41.9, 56.4 69.9, 72.2, 126.6, 127.0, 127.3, 127.6, 128.1, 128.3, 129.0, 131.7, 137.9, 145.5, 145.8, 158.7 (q, $J$ = 31.0 Hz), 164.9, 165.6, 169.0. LC–MS (ESI) purity 99%, *m/z* 509.4 $[M+H]^+$, (method d). Anal ($C_{29}H_{30}F_6N_6O_8S \times 2.3\ H_2O$) C, H, N.

#### 4.3.70. (*S*)-3-(*N*-(1-(4-Amidiniobenzylamino)-1-oxo-3-phenylpropan-2-yl)sulfamoyl)benzamidinium bis(trifluoroacetate) (70)

Yield 16%, mp 225–231 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 2.70, 2.89 (each dd, $J$ = 13.6 Hz, $J$ = 6.3 Hz, 2H), 4.00–4.07 (m, 2H), 4.20 (dd, $J$ = 16.1 Hz, $J$ = 6.3 Hz, 1H), 7.09–7.10 (m, 2H), 7.15–7.17 (m, 3H), 7.18 (d, $J$ = 8.6 Hz, 2H), 7.66 (dd, $J$ = 7.9 H, $J$ = 7.9 Hz, 1H), 7.70 (d, $J$ = 8.5 Hz, 2H), 7.84–7.86 (m, 1H), 7.94–7.96 (m, 1H), 8.00 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 8.48 (d, $J$ = 9.2 Hz, 1H), 8.57 (t, $J$ = 5.7 Hz, 1H), 9.07, 9.24, 9.31, 9.43 (each s, 8H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 38.4, 41.7, 58.1, 117.2 (q, $J$ = 279.0 Hz), 126.2, 126.6, 126.7, 127.2, 128.1, 128.2, 128.9, 129.3, 129.9, 131.2, 131.8, 136.8, 141.9, 145.3, 158.8 (q, $J$ = 31.0 Hz), 164.9, 165.5, 170.4. LC–MS (ESI) purity 99%, *m/z* 479.3 $[M+H]^+$, (method d). Anal ($C_{28}H_{28}F_6N_6O_7S \times 2.4\ H_2O$) C, H, N.

#### 4.3.71. (*R*)-3-(*N*-(1-(4-Amidiniobenzylamino)-1-oxo-3-phenylpropan-2-yl)sulfamoyl)benzamidinium bis(trifluoroacetate) (71)

Yield 22%, mp 235–237 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 2.67–2.72 (m, 1H), 2.86–2.90 (m, 1H), 4.01–4.06 (m, 2H), 4.21 (dd, $J$ = 16.1 Hz, $J$ = 6.0 Hz, 1H), 7.08–7.11 (m, 2H), 7.15–7.17 (m, 3H), 7.19 (d, $J$ = 8.5 Hz, 2H), 7.66 (dd, $J$ = 7.9 H, $J$ = 7.9 Hz, 1H), 7.69 (d, $J$ = 8.5 Hz, 2H), 7.83–7.86 (m, 1H), 7.93–7.95 (m, 1H), 7.99 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 8.49 (d, $J$ = 8.8 Hz, 1H), 8.56 (t, $J$ = 5.7 Hz, 1H), 9.07, 9.24, 9.31, 9.43 (each s, 8H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 38.5, 41.8, 58.1, 126.2, 126.6, 126.8, 127.3, 128.1, 128.3, 129.0, 129.4, 130.0, 131.3, 131.9, 136.8, 141.9, 145.4, 158.7 (q, $J$ = 31.0 Hz), 165.0, 165.5, 170.4. LC–MS (ESI) purity 99%, *m/z* 479.3 $[M+H]^+$, (method d). Anal ($C_{28}H_{28}F_6N_6O_7S \times 1.2\ H_2O$) C, H, N.

#### 4.3.72. (*S*)-3-(*N*-(1-(4-Amidiniobenzylamino)-3-benzyloxy-1-oxopropan-2-yl)sulfamoyl)benzamidinium bis(trifluoroacetate) (72)

Yield 7%, mp 207–210 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.44–3.53 (m, 2H), 4.09 (dt, $J$ = 8.2 Hz, $J$ = 6.3 Hz, 1H), 4.17, 4.30 (each dd, $J$ = 16.1 Hz, $J$ = 5.7 Hz, 2H), 4.35 (d, $J$ = 7.6 Hz, 2H), 7.22–7.24 (m, 2H), 7.27–7.35 (m, 3H), 7.39 (d, $J$ = 8.5 Hz, 2H), 7.71 (d, $J$ = 8.5 Hz, 2H), 7.77 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 8.02–8.04 (m, 1H), 8.14–8.16 (m, 1H), 8.24 (dd, $J$ = 1.6 Hz, $J$ = 1.6 Hz, 1H), 8.48 (d, $J$ = 8.5 Hz, 1H), 8.66 (t, $J$ = 6.3 Hz, 1H), 9.50, 9.54 (each s, 8H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 41.9, 56.3, 69.9, 72.2, 126.5, 126.6, 127.3, 127.6, 128.1, 128.3, 129.1, 130.1, 131.5, 132.1, 137.9, 141.9, 145.5, 145.8, 158.7 (q, $J$ = 31.0 Hz), 165.0, 165.6, 169.0. LC–MS (ESI) purity 99%, *m/z* 509.3 $[M+H]^+$, (method d). Anal ($C_{29}H_{30}F_6N_6O_8S \times 2.4\ H_2O$) C, H, N.

#### 4.3.73. (*R*)-3-(*N*-(1-(4-Amidiniobenzylamino)-3-benzyloxy-1-oxopropan-2-yl)sulfamoyl)benzamidinium bis(trifluoroacetate) (73)

Yield 14%, mp 204–205 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 3.44–3.51 (m, 2H), 4.07 (t, $J$ = 5.4 Hz, 1H), 4.17, 4.29 (dd, $J$ = 16.1 Hz, $J$ = 5.7 Hz, 2H), 4.35 (d, $J$ = 7.6 Hz, 2H), 7.20–7.22 (m, 2H), 7.25–7.33 (m, 3H), 7.38 (d, $J$ = 8.6 Hz, 2H), 7.68 (d, $J$ = 8.2 Hz, 2H), 7.75 (dd, $J$ = 7.9 Hz, $J$ = 7.9 Hz, 1H), 7.99–8.01 (m, 1H), 8.12–8.15 (m, 1H), 8.21 (dd, $J$ = 1.9 Hz, $J$ = 1.9 Hz, 1H), 8.46 (s, 1H), 8.63 (t, $J$ = 6.3 Hz, 1H), 9.29 (s, 8H). APT $^{13}$C NMR (DMSO-$d_6$): $\delta$ 41.9, 56.3, 69.9, 72.2, 126.5, 126.6, 127.3, 127.6, 128.1, 128.3, 129.1, 130.1, 131.5, 132.1, 137.9, 141.9, 145.5, 145.8, 158.7 (q, $J$ = 31.0 Hz), 165.0, 165.6, 169.0. LC–MS (ESI) purity 99%, *m/z* 509.4 $[M+H]^+$, (method d). Anal ($C_{29}H_{30}F_6N_6O_8S \times 2.7\ H_2O$) C, H, N.

### 4.4. Enzyme kinetics

#### 4.4.1. Assay conditions

The activity of bovine trypsin and of matriptase-2 in the conditioned medium of transfected HEK cells (HEK-MT2 cells) was determined by monitoring the release of 7-amino-4-methylcoumarin from the fluorogenic substrate Boc-Gln-Ala-Arg-NH-Mec. Cbz-Gly-Gly-Arg-NH-Mec was used for the determination of the activity of human thrombin. Reactions were monitored with an excitation wavelength of 340 nm and emission wavelength of 460 nm. Inhibition assays were performed in duplicate measurements with five different inhibitor concentrations. Steady-state velocities were plotted against inhibitor concentrations to obtain $IC_{50}$ values by nonlinear regression according to equation $v = v_0/(1+[I]/IC_{50})$. The $K_m$ value for bovine trypsin and Boc-Gln-Ala-Arg-NH-Mec obtained in triplicate measurements with 9 different substrate concentrations was 22.12 ± 4.01 μM. The $K_m$ value for human thrombin and Cbz-Gly-Gly-Arg-NH-Mec obtained in duplicate measurements with 12 different substrate concentrations was 40.21 ± 2.33 μM. The $K_m$ value for human matriptase-2 in the conditioned medium of HEK-MT2 cells and Boc-Gln-Ala-Arg-NH-Mec obtained in duplicate measurements with nine different substrate concentrations was 32.18 ± 2.78 μM. $K_i$ values were calculated using the equation $K_i = IC_{50}/(1+[S]/K_m)$.

#### 4.4.2. Bovine trypsin inhibition assay

Assay buffer was 50 mM Tris–HCl, 150 mM NaCl, pH 8.0. The enzyme stock solution (1 mg/mL) was prepared in 1 mM HCl, kept at 0 °C and diluted with assay buffer. A 10 mM stock solution of the fluorogenic substrate Boc-Gln-Ala-Arg-NH-Mec in DMSO was diluted with assay buffer. The final concentration of the substrate was 40 μM, of DMSO was 6% and of bovine trypsin was 12.5 ng/mL. Into each well containing 168.8 μL buffer, 11.2 μL of an inhibitor solution in DMSO and 10 μL of a substrate solution (800 μM) were added and thoroughly mixed. The reaction was performed at 25 °C, initiated by adding 10 μL of enzyme solution (250 ng/mL) and followed over 400 s.

#### 4.4.3. Human thrombin inhibition assay

Assay buffer was 50 mM Tris–HCl, 150 mM NaCl, pH 8.0. The enzyme stock solution (10000 U/mL) was prepared in water, kept

at 0 °C and diluted with assay buffer. A 10 mM stock solution of fluorogenic substrate Cbz-Gly-Gly-Arg-NH-Mec in DMSO was diluted with assay buffer. The final concentration of the substrate was 40 μM, of DMSO was 6% and of human thrombin was 2.5 U/mL. Into each well containing 173.8 μL buffer, 11.2 μL of an inhibitor solution in DMSO and 10 μL of a substrate solution (800 μM) were added and thoroughly mixed. The reaction was performed at 25 °C, initiated by adding 5 μL of enzyme solution (100 U/mL) and followed over 400 s.

#### 4.4.4. Human matriptase-2 inhibition assay

Assay buffer was 50 mM Tris–HCl, 150 mM NaCl, pH 8.0. The conditioned medium of HEK-MT2 cells was used as a source of matriptase-2 activity. The conditioned medium was collected and concentrated, and the supernatant was stored at −20 °C. After thawing, it was diluted with assay buffer (1:50) and kept at 0 °C.[38,39] A 10 mM stock solution of fluorogenic substrate Boc-Gln-Ala-Arg-NH-Mec in DMSO was diluted with assay buffer. The final concentration of the substrate was 40 μM and of DMSO was 6%. Into each well containing 143.8 μL buffer, 11.2 μL of an inhibitor solution in DMSO and 10 μL of a substrate solution (800 μM) were added and thoroughly mixed. At 37 °C the reaction was initiated by adding 35 μL of diluted conditioned medium and followed over 400 s.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.08.042.

## References and notes

1. (a) Clement, B. *Drug Metab. Rev.* **2002**, *43*, 565; (b) Ettmayer, P.; Amidon, G. L.; Clement, B.; Testa, B. *J. Med. Chem.* **2004**, *47*, 2393; (c) Havemeyer, A.; Bittner, F.; Wollers, S.; Mendel, R.; Kunze, T.; Clement, B. *J. Biol. Chem.* **2006**, *281*, 34796.
2. (a) Pinner, A. *Die Iminoäther und ihre Derivate*; Oppenheim: Germany, Berlin, 1892; (b) Roger, R.; Neilson, D. G. *Chem. Rev.* **1961**, *61*, 179.
3. (a) Leban, J.; Pegoraro, S.; Dormeyer, M.; Lanzer, M.; Aschenbrenner, A.; Kramer, B. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1979; (b) Mayence, A.; Vanden Eynde, J. J.; Krogstad, F. M.; Krogstad, D. J.; Cushion, M. T.; Huang, T. L. *J. Med. Chem.* **2004**, *47*, 2700; (c) Mayence, A.; Vanden Eynde, J. J.; LeCour, L., Jr.; Walker, L. A.; Tekwani, B. L.; Huang, T. L. *Eur. J. Med. Chem.* **2004**, *39*, 547; (d) Patrick, D. A.; Bakunov, S. A.; Bakunova, S. M.; Kumar, E. V. K. S.; Lombardy, R. J.; Jones, S. K.; Bridges, A. S.; Zhirnov, O.; Hall, J. E.; Wenzler, T.; Brun, R.; Tidwell, R. R. *J. Med. Chem.* **2007**, *50*, 2468; (e) Bakunova, S. M.; Bakunov, S.; Wenzler, T.; Barszcz, T.; Werbovetz, K. A.; Brun, R.; Tidwell, R. R. *J. Med. Chem.* **2009**, *52*, 4657.
4. (a) Wagner, G. *Folia Haematol. (Leipzig)* **1982**, *109*, 67; (b) Danilewicz, J. C.; Abel, S. M.; Brown, A. D.; Fish, P. V.; Hawkeswood, E.; Holland, S. J.; James, K.; McElroy, A. B.; Overington, J.; Powling, M. J.; Rance, D. J. *J. Med. Chem.* **2002**, *45*, 2432; (c) Hauel, N. H.; Nar, H.; Priepke, H.; Ries, U.; Stassen, J.-M.; Wienen, W. *J. Med. Chem.* **2002**, *45*, 1757; (d) Zega, A.; Mlinšek, G.; Šolmaier, T.; Trampuš-Bakija, A.; Stegnar, M.; Urleb, U. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1563; (e) Ilaš, J.; Jakopin, Ž.; Borštnar, T.; Stegner, M.; Kikelj, D. *J. Med. Chem.* **2008**, *51*, 5617; (f) Ilaš, J.; Tomašić, Kikelj, D. *J. Med. Chem.* **2008**, *51*, 2863.
5. Stürzebecher, J.; Prasa, D.; Hauptmann, J.; Vieweg, H.; Wikström, P. *J. Med. Chem.* **1997**, *40*, 3091.
6. Schärer, K.; Morgenthaler, M.; Paulini, R.; Obst-Sander, U.; Banner, D. W.; Schlatter, D.; Benz, J.; Stihle, M.; Diederich, F. *Angew. Chem., Int. Ed.* **2005**, *44*, 4400.
7. (a) Bredereck, H.; Gompper, R.; Seiz, H. *Chem. Ber.* **1957**, *90*, 1837; (b) Nii, Y.; Okano, K.; Kobayashi, S.; Ohno, M. *Tetrahedron Lett.* **1979**, *27*, 2517.
8. (a) Zablocki, J. A.; Masateru, M.; Garland, R. B.; Daisy, P.; Schretzman, L.; Rao, S. N.; Lindmark, R. J.; Panzer-Knodle, S. G.; Nicholson, N. S.; Taite, B. B.; Salyers, A. K.; King, L. W.; Campion, J. G.; Feigen, L. P. *J. Med. Chem.* **1993**, *36*, 1811; (b) Hayashi, Y.; Katada, J.; Harada, T.; Tachiki, A.; Iijima, K.; Takiguchi, Y.; Muramatsu, M.; Miyazaki, H.; Asari, T.; Okazaki, T.; Sato, Y.; Yasuda, E.; Yano, M.; Uno, I.; Ojima, I. *J. Med. Chem.* **1998**, *41*, 2345.
9. Cui, J.; Marankan, F.; Fu, W.; Crich, D.; Mesecar, A.; Johnson, M. E. *Bioorg. Med. Chem.* **2002**, *10*, 41.
10. (a) Su, T.; Wu, Y.; Doughan, B.; Jia, Z. J.; Woolfrey, J.; Huang, B.; Wong, P.; Park, G.; Sinha, U.; Scarborough, R. M.; Zhu, B.-yan *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2947; (b) Rumthao, S.; Lee, O.; Sheng, Q.; Fu, W.; Mulhearn, D. C.; Crich, D.; Mesecar, A. D.; Johnson, M. E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5165.
11. Gabriel, B.; Stubbs, M. T.; Bergner, A.; Hauptmann, J.; Bode, W.; Stürzebecher, J.; Moroder, L. *J. Med. Chem.* **1998**, *41*, 4240.
12. (a) Levin, J. I.; Turos, E.; Weinreb, S. M. *Synth. Commun.* **1982**, *12*, 989; (b) Garigipati, R. S. *Tetrahedron Lett.* **1990**, *31*, 1969.
13. Aslanian, R.; Brown, J. E.; Shih, N.-Y.; Wa Mutahi, M.; Green, M. J.; She, S.; Prado, M. D.; West, R.; Hey, J. *Bioorg. Med. Chem. Lett* **1998**, *8*, 2263.
14. Boeré, R. T.; Oakley, R. T.; Reed, R. W. *J. Organomet. Chem.* **1987**, *331*, 161.
15. Reid, C. M.; Ebikeme, C.; Barrett, M. P.; Patzewitz, E.-M.; Müller, S.; Robins, D. J.; Sutherland, A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5399.
16. Vogler, M.; Koert, U.; Dorsch, D.; Gleitz, J.; Raddatz, P. *Synlett* **2003**, 1683.
17. (a) Eloy, F.; Lenaers, R. *Chem. Rev.* **1962**, *62*, 155; (b) Anbazhagan, M.; Boykin, D. W.; Stephens, C. E. *Synthesis* **2003**, 2467; (c) Nadrah, K.; Sollner Dolenc, M. *Synlett* **2007**, 1257.
18. Cesar, J.; Nadrah, K.; Sollner Dolenc, M. *Tetrahedron Lett.* **2004**, *45*, 7445.
19. Judkins, B. D.; Allen, D. G.; Cook, T. A.; Evans, B.; Sardharwala, T. E. *Synth. Commun.* **1996**, *26*, 4351.
20. (a) Ismail, M. A.; Brun, R.; Easterbrook, J. D.; Tanious, F. A.; Wilson, W. D.; Boykin, D. W. *J. Med. Chem.* **2003**, *46*, 4761; (b) Ismail, M. A.; Arafa, R. K.; Brun, R.; Wenzler, T.; Miao, Y.; Wilson, W. D.; Generaux, C.; Bridges, A.; Hall, J. E.; Boykin, D. W. *J. Med. Chem.* **2006**, *49*, 5324; Ismail, M. A.; Arafa, R. K.; Wenzler, T.; Brun, R.; Tanious, F. A. W.; David Wilson, W. D.; Boykin, D. W. *Bioorg. Med. Chem.* **2008**, *16*, 683.
21. Schweinitz, A.; Stürzebecher, A.; Stürzebecher, U.; Schuster, O.; Stürzebecher, J.; Steinmetzer, T. *Med. Chem.* **2006**, *2*, 349.
22. (a) Stürzebecher, J.; Vieweg, H.; Steinmetzer, T.; Schweinitz, A.; Stubbs, M. T.; Renatus, M.; Wikström, P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3147; (b) Verner, E.; Katz, B. A.; Spencer, J. R.; Allen, D.; Hataye, J.; Hruzewicz, W.; Hui, H. C.; Kolesnikov, A.; Li, Y.; Luong, C.; Martelli, A.; Radika, K.; Rai, R.; She, M.; Shrader, W.; Sprengeler, P. A.; Trapp, S.; Wang, J.; Young, W. B.; Mackman, R. L. *J. Med. Chem.* **2001**, *44*, 2753; (c) Schweinitz, A.; Steinmetzer, T.; Banke, I. J.; Arlt, M. J.; Stürzebecher, A.; Schuster, O.; Geissler, A.; Giersiefen, H.; Zeslawska, E.; Jacob, U.; Krüger, A.; Stürzebecher, J. *J. Biol. Chem.* **2004**, *279*, 33613.
23. (a) Steinmetzer, T.; Schweinitz, A.; Stürzebecher, A.; Dönnecke, D.; Uhland, K.; Schuster, O.; Steinmetzer, P.; Müller, F.; Friedrich, R.; Than, M. E.; Bode, W.; Stürzebecher, J. *J. Med. Chem.* **2006**, *49*, 4116; (b) Steinmetzer, T.; Dönnecke, D.; Korsonewski, M.; Neuwirth, C.; Steinmetzer, P.; Schulze, A.; Saupe, S. M.; Schweinitz, A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 67; (c) Schweinitz, A.; Dönnecke, D.; Ludwig, A.; Steinmetzer, P.; Schulze, A.; Kotthaus, J.; Wein, S.; Clement, B.; Steinmetzer, T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1960; (d) Hammami, M.; Rühmann, E.; Maurer, E.; Heine, A.; Gütschow, M.; Klebe, G.; Steinmetzer, T. *Med. Chem. Commun.* **2012**, *3*, 807.
24. Huang, T. L.; Bacchi, C. J.; Kode, N. R.; Zhang, Q.; Wang, G.; Yartlet, N.; Rattendi, D.; Londono, I.; Mazumder, L.; Vanden Eynde, J. J.; Mayence, A.; Donkor, I. O. *Int. J. Antimicrob. Agents* **2007**, *30*, 555.
25. Huang, T. L.; Vanden Eynde, J. J.; Mayence, A.; Collins, M. S.; Cushion, M. T.; Rattendi, D.; Londono, I.; Mazumder, L.; Bacchi, C. J.; Yarlett, N. *Bioorg. Med. Chem. Lett* **2009**, *19*, 5884.
26. Huang, T. L.; Tao, B.; Quarshie, Y.; Queener, S. F.; Donkor, I. O. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2679.
27. Peterlin-Mašič, L. *Curr. Med. Chem.* **2006**, *13*, 3627.
28. (a) Weller, T.; Alig, L.; Beresini, M.; Blackburn, B.; Bunting, S.; Hadváry, P.; Hürzeler Müller, M.; Knopp, D.; Levet-Trafit, B.; Lipary, M. T.; Modi, N. B.; Müller, M.; Refino, C. J.; Schmitt, M.; Schönholzer, P.; Weiss, S.; Steiner, B. *J. Med. Chem.* **1996**, *39*, 3139; (b) Kitamura, S.; Fukushi, H.; Miyawaki, T.; Kawamura, M.; Terashita, Z.-I.; Sugihara, H.; Naka, T. *Chem. Pharm. Bull.* **2001**, *49*, 258.
29. Pinto, D. J. P.; Smallheer, J. M.; Cheney, D. L.; Knabb, R. M.; Wexler, R. R. *J. Med. Chem.* **2010**, *53*, 6243.
30. Stürzebecher, A.; Dönnecke, D.; Schweinitz, A.; Schuster, O.; Steinmetzer, P.; Stürzebecher, U.; Kotthaus, J.; Clement, B.; Stürzebecher, J.; Steinmetzer, T. *ChemMedChem* **2007**, *2*, 1043.
31. Saupe, S. M.; Steinmetzer, T. *J. Med. Chem.* **2012**, *55*, 1171.
32. Straub, A.; Roehrig, S.; Hillisch, A. *Angew. Chem., Int. Ed.* **2011**, *50*, 4574.
33. Gustafsson, D.; Bylund, R.; Antonsson, T.; Nilsson, I.; Nyström, J.-E.; Eriksson, U.; Bredberg, U.; Teger-Nilsson, A.-C. *Nat. Rev.* **2004**, *3*, 649.
34. Dullweber, F.; Stubbs, M. T.; Musil, D.; Stürzebecher, J.; Klebe, G. *J. Mol. Biol.* **2001**, *313*, 593.
35. Di Fenza, A.; Heine, A.; Koert, U.; Klebe, G. *ChemMedChem* **2007**, *2*, 297.
36. Velasco, G.; Santiago, C.; Victor, Q.; Luis, M. S.; Carlos, L. *J. Biol. Chem.* **2002**, *177*, 376371.
37. Béliveau, F.; Désilets, A.; Leduc, R. *FEBS J.* **2009**, *276*, 2213.
38. Stirnberg, M.; Maurer, E.; Horstmeyer, A.; Kolp, S.; Frank, S.; Bald, T.; Arenz, K.; Janzer, A.; Prager, K.; Wunderlich, P.; Walter, J.; Gütschow, M. *Biochem. J.* **2010**, *430*, 87.
39. Sisay, M. T.; Steinmetzer, T.; Stirnberg, M.; Maurer, E.; Hammami, M.; Bajorath, J.; Gütschow, M. *J. Med. Chem.* **2010**, *53*, 5523.

40. (a) Shi, Y. E.; Torri, J.; Yieh, L.; Wellstein, A.; Lippman, M. E.; Dickson, R. B. *Clin. Cancer Res.* **1993**, *53*, 1409; (b) Lin, C. Y.; Anders, J.; Johnson, M.; Sang, Q. A.; Dickson, R. B. *J. Biol. Chem.* **1999**, *174*, 18231; Oberst, M. D.; Johnson, M. D.; Dickson, R. B.; Lin, C.-Y.; Singh, B.; Stewart, M.; Williams, A.; Al-Nafussi, A.; Smyth, J. F.; Gabra, H.; Sellar, G. C. *Clin. Cancer Res.* **2002**, *8*, 1101.
41. Ramsay, A. J.; Reid, J. C.; Velasco, G.; Quigley, J. P.; Hopper, J. D. *Front. Biosci.* **2008**, *13*, 569.
42. (a) Parr, C.; Sanders, A. J.; Davies, G.; Martin, T.; Lane, J.; Mason, M. D.; Mansel, R. E.; Jiang, W. G. *Clin. Cancer Res.* **2007**, *13*, 3568; (b) Sanders, A. J.; Parr, C.; Martin, T. A.; Lane, J.; Mason, M. D.; Jiang, W. G. *J. Cell. Physiol.* **2008**, *216*, 780; (c) Sanders, A. J.; Webb, S. L.; Parr, C.; Mason, M. D.; Jiang, W. G. *Anticancer Agents Med. Chem.* **2010**, *10*, 64.
43. (a) Finberg, K. E.; Heeney, M. M.; Campagna, D. R.; Aydınok, Y.; Pearson, H. A.; Hartman, K. R.; Mayo, M. M.; Samuel, S. M.; Strouse, J. J.; Markianos, K.; Andrews, N. C.; Fleming, M. D. *Nat. Genet.* **2008**, *40*, 569; (b) Du, X.; She, E.; Gelbart, T.; Truska, J.; Lee, P.; Xia, Y.; Khovananth, K.; Mudd, S.; Mann, N.; Moresco, E. M. Y.; Beutler, E.; Beutler, B. *Science* **2008**, *320*, 1088.
44. (a) Silvestri, L.; Pagani, A.; Nai, A.; De Domenico, I.; Kaplan, J.; Camaschella, C. *Cell Metab.* **2008**, *8*, 502; (b) Truska, J.; Gelbart, T.; Peng, H.; Beutler, E.; Beutler, B.; Lee, P. *Br. J. Haematol.* **2009**, *147*, 571; (c) Lee, P. *Acta Haematol.* **2009**, *122*, 87; (d) Silvestri, L.; Guillem, F.; Pagani, A.; Nai, A.; Oudin, C.; Silva, M.; Toutain, F.; Kannengiesser, C.; Beaumont, C.; Camaschella, C.; Grandchamp, B. *Blood* **2009**, *113*, 5605.
45. (a) Gante, J. *Angew. Chem., Int. Ed.* **1994**, *33*, 1669; (b) Obreza, A.; Gobec, S. *Curr. Med. Chem.* **2004**, *11*, 3263.
46. Dosa, S.; Daniels, J.; Gütschow, M. *J. Heterocycl. Chem.* **2011**, *48*, 407.
47. Böhm, M.; Stürzebecher, J.; Klebe, G. *J. Med. Chem.* **1999**, *42*, 458.
48. Bode, W.; Mayr, I.; Baumann, U.; Huber, R.; Stone, S. R.; Hofsteenge, J. *EMBO J.* **1989**, *8*, 3567.
49. Maurer, E.; Sisay, M. T.; Stirnberg, M.; Steinmetzer, T.; Bajorath, J.; Gütschow, M. *ChemMedChem* **2012**, *7*, 68.
50. Bajusz, S.; Barabás, E.; Tolnay, P.; Széll, E.; Bagdy, D. *Int. J. Pept. Protein Res.* **1978**, *12*, 217.