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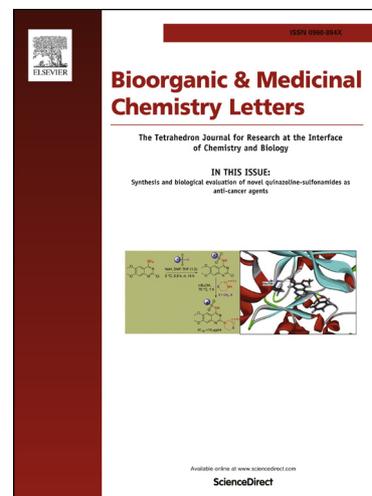
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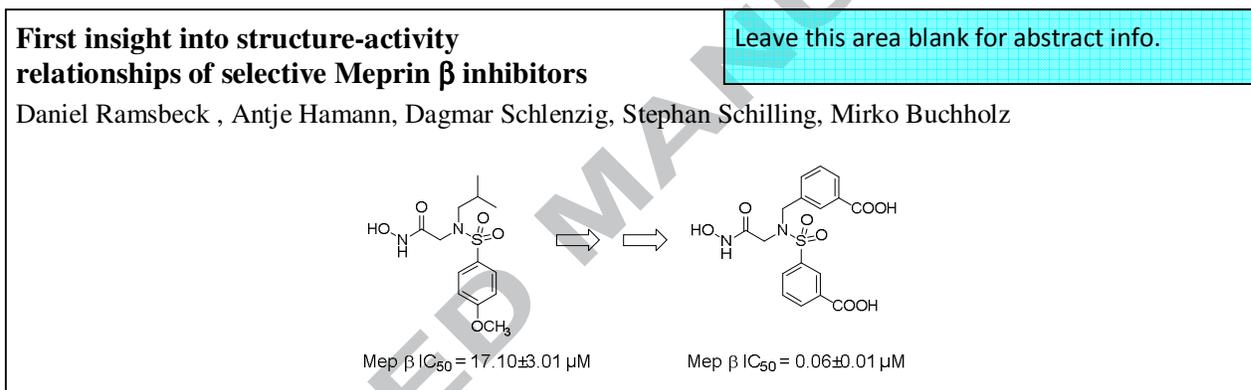
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Graphical Abstract



Keywords

Meprin β , hydroxamic acid, sulfonamides, astacins

Abstract

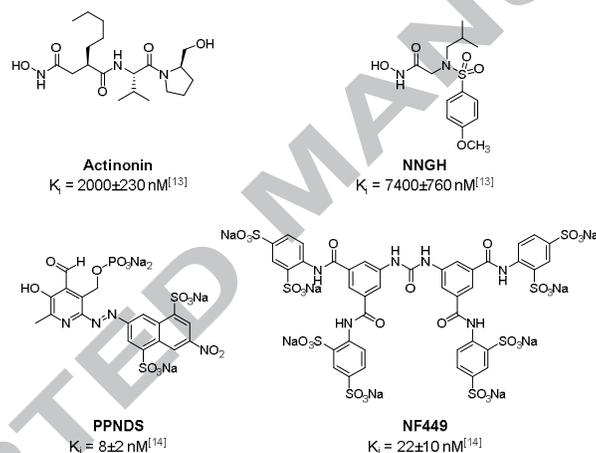
The astacin proteases meprin α and β are emerging drug targets for treatment of disorders such as kidney failure, fibrosis or inflammatory bowel disease. However, there are only few inhibitors of both proteases reported to date. Starting from NNGH as lead structure, a detailed elaboration of the structure-activity relationship of meprin β inhibitors was performed, leading to compounds with activities in the lower nanomolar range. Considering the preference of meprin β for acidic residues in the P1' position, the compounds were optimized. Acidic modifications induced potent inhibition and >100-fold selectivity over other structurally related metalloproteases such as MMP-2 or ADAM10.

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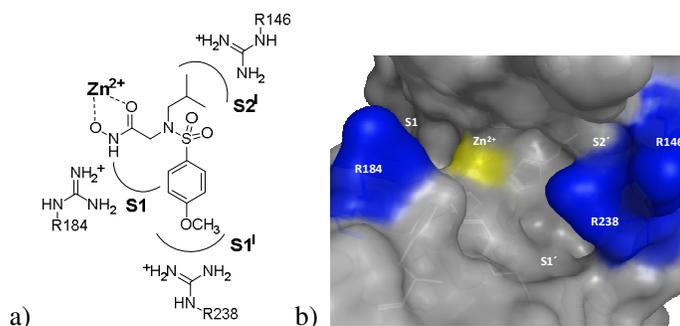
Compelling evidence suggests the endoproteases meprin α and β as novel drug targets^[1,2]. Due to their procollagenase activity, meprins have been linked to disorders that involve collagen deposition, e.g. skin or lung fibrosis and keloids^[1,3]. Moreover, meprins are associated with nephritis, cancer and inflammatory bowel disease^[4,5] and in particular meprin β has also been shown to act as a β -secretase independent from BACE1^[6-8]. The meprins belong to the astacins, a subfamily of the metzincin superfamily of proteolytic enzymes. Hence, they are structurally and functionally related to well-characterized metalloproteases such as MMPs or ADAMs^[9]. Meprin β exhibits an unique substrate recognition pattern, preferring acidic amino acids in P1 to P2'-position^[10]. This unusual substrate specificity is caused by a clustering of arginine residues within the active site (figure 2b), which is not observed with other metzincins and astacins, e.g. meprin α or BMP-1^[11,12]. On our quest to decipher the role of meprin activity in these disorders, we aimed here at the discovery of selective meprin β inhibitors.

Figure 1: Inhibition constants of known meprin β inhibitors



Only few inhibitors of meprins have been reported to date (figure 1). Among those, the naturally occurring compound Actinonin proved to be the most potent inhibitor of both, meprin α and β . Some other inhibitors of MMPs or ADAMs also exhibited inhibitory activity on meprins, but displayed much lower potency than Actinonin^[13]. More recently, the compounds PPNDS and NF449 have been reported as very potent and partially selective inhibitors of meprin β ^[14].

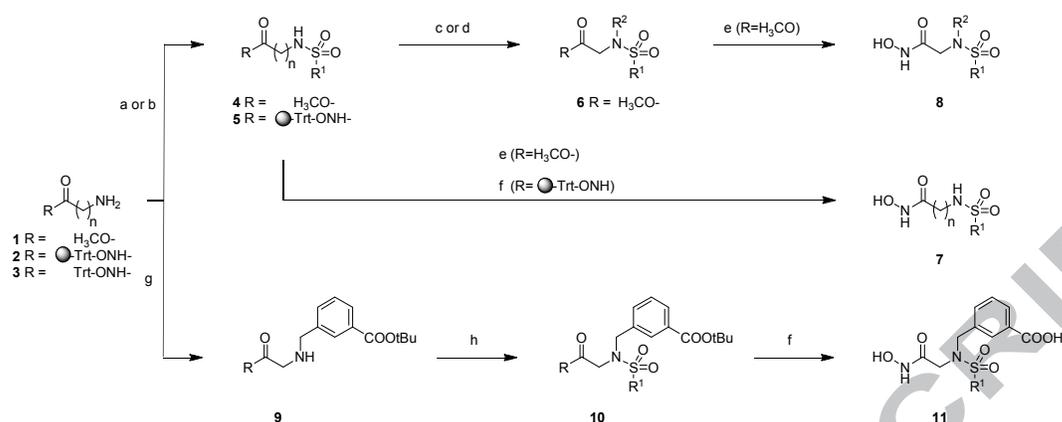
Figure 2: Schematic depiction of the assumed binding mode to meprin β (left); active site of meprin β (right)



Despite the higher potency of Actinonin, NNGH (N-Isobutyl-N-(4-methoxyphenylsulfonyl)glycyl hydroxamic acid)^[13] served as our lead structure for the development of meprin β inhibitors, due to the easy chemical accessibility of this well-known sulfonamide MMP-inhibitor scaffold. Metzincin-metalloprotease inhibitors with sulfonamide-scaffold bind to their target enzymes as depicted in figure 2a^[15,16]. A similar binding mode of NNGH to the active site of meprin β was assumed. Thus, a ligand-based compound optimization was performed, with modifications of the moieties targeting the individual subsites of meprin β , i.e. arginine residues shaping S1, S1' or S2'.

The compounds have been synthesized using glycine or β -alanine derivatives as precursors (scheme 1). For the synthesis of the secondary sulfonamides **7a-w**, either glycine or β -alanine methyl ester was sulfamoylated with the respective sulfamoyl chloride, followed by conversion of the ester to the hydroxamic acid by means of hydroxylamine under microwave irradiation. Some compounds were synthesized on solid support using chlorotriptyl-hydroxylamine resin, which was preloaded with Fmoc-glycine. After Fmoc-removal under standard conditions, the amino group was sulfamoylated, followed by cleavage from the resin using trifluoroacetic acid in dichloromethane. For the synthesis of the tertiary sulfonamides **8a-m**, sulfonamides **4** were treated with alcohols under Mitsunobu conditions or were alkylated using alkyl or benzyl halides under microwave irradiation. The resulting glycine-sulfonamide methyl esters **6** were transformed to the corresponding hydroxamic acids **8** using hydroxylamine hydrochloride under microwave irradiation. Inhibitors carrying an N-(3-carboxybenzyl) moiety (**11a-g**) were synthesized by reductive amination of **3** with tert-Butyl-3-formylbenzoate, followed by sulfamoylation under microwave irradiation and subsequent deprotection.

Scheme 1: General synthesis scheme of the sulfonamide hydroxamic acids

**Conditions:**

a) R²-SO₂Cl, TEA, THF, rt; b) R¹-SO₂Cl, K₂CO₃, H₂O/dioxane, rt; c) R²-OH, DIAD, PPh₃, THF, 0°-rt; d) R²-Hal, K₂CO₃, MeCN, MW, 120°C; e) NH₂OH·HCl, KOH, MeOH, MW 80°C(f) TFA/DCM (1:1 v/v), TIS, rt; g) *tert*-butyl 3-formylbenzoate, NaBH₄, MeOH, rt; h) R¹-SO₂Cl, THF, TEA, MW 100°C

In the first optimization step the sulfonamide residue was modified. This residue addresses the S1' pocket of the metzincin proteases and is the major determinant of compound selectivity. Exploration of the S1'-substituent was performed by introducing diverse aromatic secondary sulfonamide moieties (table 1). However, the impact of most substituents on the activity was very low and only the introduction of acidic functional groups (**7q-s**) led to an increased activity compared to NNGH. A very similar inhibitory potency was also achieved with the respective β -alanine derivatives **7v-w**. These results nicely correspond to the preference of meprin β for acidic amino acids in P1' position of the substrates, which is determined by ionic interactions with positively charged arginine R238 forming the S1'-subpocket (figure 2). Because halophenols have been reported as carboxylic acid bioisosters^[17], a dichlorophenol was introduced (**7s**), which exhibited similar inhibitory potency as the carboxylic acid derivatives. Interestingly, the introduction of an additional methylene group between the aromatic ring and the carboxylic acid (**7t**) led to a loss of activity compared to **7q** and **r**. This suggests a steric hindrance within the S1'-pocket, ruling out an interaction of the carboxylic acid with the S1'-arginine residue.

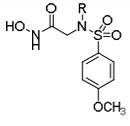
Table 1: Inhibition of meprin β by secondary sulfonamides

	n	R ¹	R ²	R ³	R ⁴	R ⁵	IC ₅₀ (μ M)
7a	1	H	H	OCH ₃	H	H	9.02±0.30
7b	1	H	OCH ₃	H	H	H	18.85±0.35
7c	1	H	OCH ₃	OCH ₃	H	H	26.90±9.62
7d	1	H	-OCH ₂ CH ₂ O-	H	H	H	7.42±0.03
7e	1	H	F	OCH ₃	H	H	6.19±0.80
7f	1	H	H	OPh	H	H	18.80±2.55
7g	1	OCH ₃	H	CH ₃	H	H	25.70±0.28

7h	1	CH ₃	H	CH ₃	H	H	14.77±0.78
7i	1	H	H	Cl	H	H	17.17±2.57
7j	1	H	H	F	H	H	14.32±0.59
7k	1	H	F	H	H	CH ₃	17.97±1.25
7l	1	H	CF ₃	Cl	H	H	10.26±1.12
7m	1	H	H	Ph	H	H	3.85±0.65
7n	1	H	H	Ph(<i>p</i> -OCH ₃)	H	H	4.42±0.18
7o	1	H	H	Ph(<i>p</i> -F)	H	H	7.31±1.06
7p	1	H	H	Ph(<i>p</i> -Cl)	H	H	5.65±0.66
7q	1	H	H	COOH	H	H	0.41±0.01
7r	1	H	COOH	H	H	H	0.31±0.01
7s	1	H	Cl	OH	Cl	H	0.43±0.08
7t	1	H	H	CH ₂ COOH	H	H	5.03±0.26
7u	2	H	H	COOH	H	H	0.34±0.04
7v	2	H	COOH	H	H	H	0.22±0.02
7w	2	H	Cl	OH	Cl	H	0.40±0.13

Next, the impact of addressing the S2'-subpocket of meprin β was evaluated (table 2). For this purpose, the 4-methoxysulfonamide moiety was kept constant and several alkyl- and arylalkyl substituents were introduced. The inhibitory potency of the synthesized compounds (**8a-i**) was virtually not affected by small alkyl or larger arylalkyl moieties. In all cases except **8h**, the activity was lower compared to NNGH. Therefore, attention was drawn again on acidic sidechains. The activity could be improved by the introduction of an acetic acid, propionic acid and a *p*-carboxybenzyl sidechain (**8j-l**), and was apparently dependent on the spacer length between the nitrogen and the carboxylic acid. The introduction of an *m*-carboxybenzyl sidechain (**8m**) led to a boost of activity, triggering an about 300-fold improved potency compared to NNGH. This suggests an improved orientation of the carboxylic acid towards arginine R146 shaping the S2'-pocket (figure 2), enabling the proper formation of an ionic interaction.

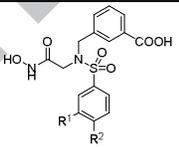
Table 2: Inhibition of meprin β by tertiary sulfonamides

	R	IC ₅₀ (μM)
NNGH	-CH ₂ CH(CH ₃) ₂	17.10±3.01
8a	-CH ₃	32.53±4.22
8b	-CH ₂ CH ₃	31.20±3.22
8c	-(CH ₂) ₃ CH ₃	37.50±16.89
8d	-CH ₂ CH ₂ CH(CH ₃) ₂	38.80±2.36
8e	-CH ₂ Ph	40.47±3.40
8f	-CH ₂ Ph(<i>p</i> -OCH ₃)	34.95±18.88

8g	-CH ₂ Ph(3,4OCH ₂ O)	1.93±0.21
8h	-CH ₂ CH ₂ Ph	42.90±4.00
8i	-CH ₂ -(2-Norbornyl)	43.87±2.08
8j	-CH ₂ COOH	5.98±0.37
8k	-CH ₂ CH ₂ COOH	2.55±0.48
8l	-CH ₂ Ph(<i>p</i> -COOH)	0.98±0.05
8m	-CH ₂ Ph(<i>m</i> -COOH)	0.06±0.001

Since the introduction of the *m*-carboxybenzyl side chain led to a significant increase of activity, this moiety was kept constant and the SAR of the S1'-substituent was re-evaluated. Caused by the additional S2'-interaction of the acidic side chain, all compounds (**11a-e**, table 3) exhibit increased activities compared to the respective secondary sulfonamides. However, only the introduction of a benzodioxolane led to an IC₅₀-value in the lower nanomolar range, similar as observed with **8m** before. Interestingly, in contrast to the secondary sulfonamide derivatives **7q** and **r**, a significant difference in the activity of the *p*- and *m*-carboxyaryl-sulfonamide was observed. For the respective tertiary sulfonamides **11f** and **g** a gain of activity was just observed by introduction of the *m*-carboxylic acid (**11g**). This could be driven by a slightly shifted binding mode due to the S2'-interaction, leading to a different positioning within the S1'-subpocket that rules out the proper interaction of arginine R238 with the *p*-carboxylic acid of **11f**.

Table 3: Inhibition of meprin β by *m*-carboxybenzyl substituted sulfonamides

	R ¹	R ²	IC ₅₀ (μM)
11a	-OCH ₂ O-		0.27±0.02
11b	F	OCH ₃	0.65±0.06
11c	H	OCF ₃	0.70±0.08
11d	H	Ph	1.08±0.45
11e	H	F	1.06±0.06
11f	H	COOH	0.32±0.02
11g	COOH	H	0.06±0.01

Numerous hydroxamate-based MMP inhibitors have been tested in model systems and clinical trials during the past years. However, most of them have been withdrawn due to toxicity issues. The reason of most of those effects are interactions not only with the respective target enzyme, but also with off-targets, i.e. other metalloproteases^[18]. Thus, the selectivity of novel metalloprotease inhibitors should

be treated with special regards. To verify the selectivity profile of the most potent novel meprin β inhibitors (**8m** and **11g**), the inhibitory activity towards selected metzincin metalloproteases, i.e. meprin α , MMP2, MMP9, MMP13, ADAM10 and ADAM17, has been evaluated.

Table 4: Inhibition data of selected metalloproteases

Protease	8m IC ₅₀ (μ M)	11g IC ₅₀ (nM)
Meprin β	0.06 \pm 0.001	0.06 \pm 0.01
Meprin α	3.29 \pm 0.41	27.35 \pm 3.04
MMP2	0.002 \pm 0.0001	n.d.*
MMP9	0.05 \pm 0.001	n.d.*
MMP13	0.0003 \pm 0.000003	n.d.*
ADAM10	11.70 \pm 1.13	n.d.*
ADAM17	1.24 \pm 0.01	n.d.*

*n.d. (not determined) – no inhibition @ 200 μ M inhibitor detected

To our surprise, **8m** is not only a potent inhibitor of meprin β , but also a very potent inhibitor of MMP2, 9 and 13. This might be due to the p-methoxyarylsulfonamide targeting the S1'-subpocket. As mentioned above, this residue determines the compound selectivity within the metzincin-superfamily and in particular the p-methoxyarylsulfonamide can be found in several non-specific metalloprotease inhibitors, e.g. NNGH^[19]. Nevertheless, this compound exhibits a good selectivity for the inhibition of meprin β over meprin α by a factor of 60. The introduction of the m-carboxyphenylsulfonamide (**11g**) significantly influenced the selectivity profile of the inhibitor. While the activity against meprin β was not affected, the activity against meprin α dropped by a factor 7 compared to **8m**, leading to a 500-fold selectivity for the inhibition of meprin β over α . Additionally, the activity against the other metalloproteases was almost completely abolished. Thus, the acidic functionality targeting the S1'-pocket is not only required for high potency against meprin β , but also leads to an excellent selectivity over other metzincins, that have been investigated so far. Thus, this enhanced selectivity renders **11g** as a promising lead compound for further development of selective meprin β inhibitors.

In summary, a first insight into SAR of meprin β inhibitors could be gained, yielding compounds with inhibitory activity in the lower nanomolar range. Furthermore, selectivity could be achieved against the isoform meprin α and also against selected structurally related metalloproteases of the metzincin-superfamily. However, additional studies, e.g. docking experiments or co-crystallization with meprin β , should give a better insight into the binding mode of those inhibitors enabling further optimization of the compounds.

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Supplementary data

Supplementary data (experimental procedures and spectroscopic characterization data of the compounds) associated with this article can be found, in the online version.

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