Bioorganic & Medicinal Chemistry Letters 27 (2017) 2428–2431

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

First insight into structure-activity relationships of selective meprin β inhibitors

Daniel Ramsbeck *, Antje Hamann, Dagmar Schlenzig, Stephan Schilling, Mirko Buchholz *

Fraunhofer Institute for Cell Therapy and Immunology IZI, Department of Drug Design and Target Validation MWT, Biocenter, Weinbergweg 22, 06120 Halle (Saale), Germany

ARTICLE INFO

Article history:
Received 15 February 2017
Revised 3 April 2017
Accepted 3 April 2017
Available online 5 April 2017

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.

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 (Fig. 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.

Only few inhibitors of meprins have been reported to date (Fig. 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]

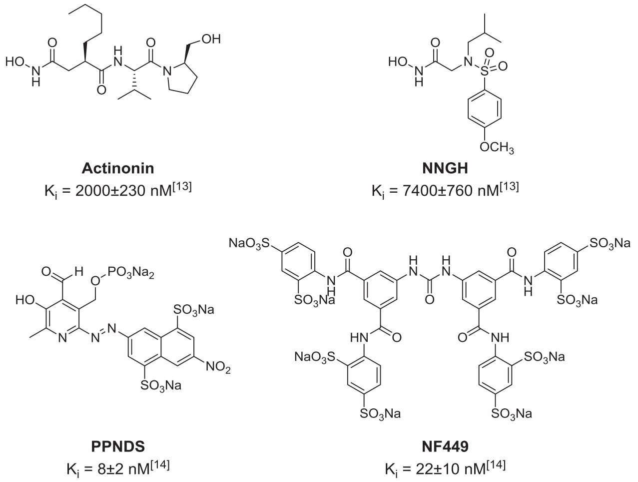


Fig. 1. Inhibition constants of known meprin β inhibitors.

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 Fig. 2a.[15,16] A similar binding mode of NNGH to the active site of meprin β was assumed. Thus, a ligand-based

---

* Corresponding authors.

E-mail addresses: daniel.ramsbeck@izi.fraunhofer.de (D. Ramsbeck), mirko.buchholz@izi.fraunhofer.de (M. Buchholz).

http://dx.doi.org/10.1016/j.bmcl.2017.04.012

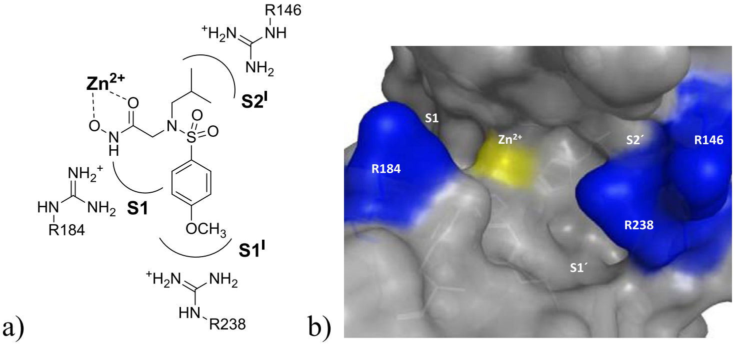
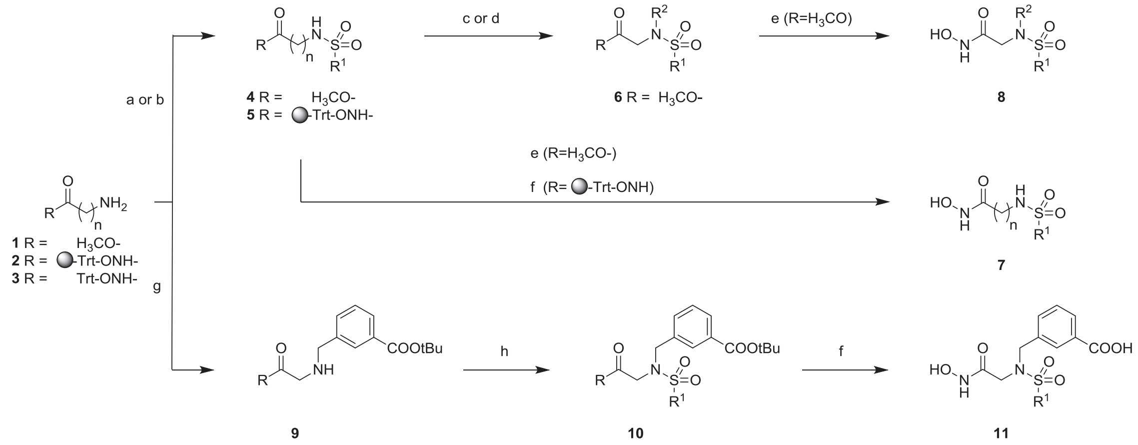


Fig. 2. Schematic depiction of the assumed binding mode to meprin β (left); active site of meprin β (right).



Conditions:
a) R^2-SO$_2$Cl, TEA,THF, rt; b) R^1-SO$_2$Cl, K$_2$CO$_3$, H$_2$O/dioxane, rt; c) R^2-OH, DIAD, PPh$_3$, THF, 0°-rt; d) R^2-Hal, K$_2$CO$_3$, MeCN, MW, 120°C; e) NH$_2$OH*HCl, KOH, MeOH, MW 80°Cf) TFA/DCM (1:1 v/v), TIS, rt; g) *tert*-butyl 3-formylbenzoate, NaBH$_4$, MeOH, rt; h) R^1-SO$_2$Cl, THF, TEA, MW 100°C

Scheme 1. General synthesis scheme of the sulfonamide hydroxamic acids.

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 chlorotrityl-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.

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 **7u**–**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 (Fig. 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.

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 **8g**, 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