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New furin inhibitors based on weakly basic amidinohydrazones

Frank Sielaff[a], Manuel E. Than[b], Dorian Bevec[c], Iris Lindberg[d], Torsten Steinmetzer[a,*]

[a] Department of Pharmaceutical Chemistry, Philipps University Marburg, Marbacher Weg 6, D-35032 Marburg, Germany
[b] Leibniz Institute for Age Research—Fritz Lipmann Institute (FLI), Protein Crystallography Group, Beutenbergstrasse 11, 07745 Jena, Germany
[c] mondoBIOTECH Holding AG, Das Kloster, Mürgstrasse 18, CH-6370 Stans, Switzerland
[d] Department of Anatomy and Neurobiology, University of Maryland-Baltimore, Baltimore, MD 21201, USA

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ABSTRACT

A novel series of amidinohydrazone-derived furin inhibitors was prepared; the most potent compounds **17** and **21** inhibit furin with K_i values of 0.46 and 0.59 μM, respectively. In contrast to inhibitor **17**, which still contains a guanidino residue, compound **21** possesses only weakly basic amidinohydrazone groups.

Furin is a type-I transmembrane protein which contains a Ca^{2+}-dependent subtilisin-like serine protease domain. It is ubiquitously distributed in human tissues and is the best characterised member of the family of proprotein convertases (PCs), which convert numerous precursors of secreted proteins to their active forms. Various studies have confirmed that furin plays a crucial role in many bacterial and viral diseases, tumorigenesis, neurodegenerative disorders and diabetes.[1,2] Furin possesses a strong preference for substrates with the multibasic cleavage motif Arg-X-Arg/Lys-Arg↓-X. In addition to various types of peptidic substrate-analogues,[3–5] potent non-peptidic furin inhibitors based on guanylated 2,5-dideoxystreptamines[6] have also been described. Recently, we have designed a series of highly potent peptidomimetic furin inhibitors which contain a 4-amidinobenzylamide group as the P1 residue. Using a cell-based assay we could demonstrate that these inhibitors are able to reduce the cleavage of the hemagglutinin precursor HA0 in H7N1 fowl plague viruses.[7] Correct cleavage of the HA0 precursor is a crucial step during an influenza virus infection.[8]

In parallel to the design of these inhibitors we screened various compounds available to us for furin inhibition and could identify a bis-(amidinohydrazone)-derivative **1** with a K_i value of 1.82 μM. This compound and several close analogues were originally described for the treatment of trypanosomiasis[9] and for inflammation.[10] Interestingly, there already exists an approved amidinohydrazone-based drug used for the treatment of hypertension, guanabenz.[11] Furthermore, CNI-1493, an anti-inflammatory and anti-parasitic compound that contains four amidinohydrazone groups, reached phase II clinical trials for the treatment of Crohn's disease.[12–14] Very recently, in parallel to our work, a related amidinohydrazone-derived furin inhibitor **2** was identified by HTS.[15] Such amidinohydrazones have a significantly decreased basicity compared to other furin inhibitors, which often contain strongly basic guanidino or amidino groups. For example, we have calculated[16] a pK_a of 8.03 for the amidinohydrazone group of inhibitor **1**, which is similar to the value of 8.1 reported for the orally available guanabenz.[17]

After identification of **1** we prepared several analogues with one or two amidinohydrazone groups by treatment of commercially available carbonyl compounds with aminoguanidine (Table 1). In addition, the known inhibitor **2**[15] was synthesized as a reference compound. For this inhibitor we found a similar potency (K_i = 25 μM) as described in the literature. In contrast, the mono-amidinohydrazones **3** and **4** derived from benzaldehyde and benzophenone, as well as the acylated analogue **5** obtained from reaction with benzoyl chloride showed poor inhibition (K_i >250 μM). Introduction of a second amidinohydrazone group in the meta and para position resulted in improved affinity, whereas both acylated aminoguanidines **8** and **11** were less active. Bis-amidinohydrazone **12** derived from 1,3-indandione and **13** obtained from 4,4′-diacyldiphenylether inhibit furin with K_i values >15 μM and were not further modified.

From the X-ray structure of furin in complex with the irreversible inhibitor decanoyl-Arg-Val-Lys-Arg-chloromethyl ketone it is known that furin has an unusually acidic active site explaining

* Corresponding author. Tel.: +49 6421 2825900; fax: +49 6421 2825901.
E-mail address: steinmetzer@staff.uni-marburg.de (T. Steinmetzer).

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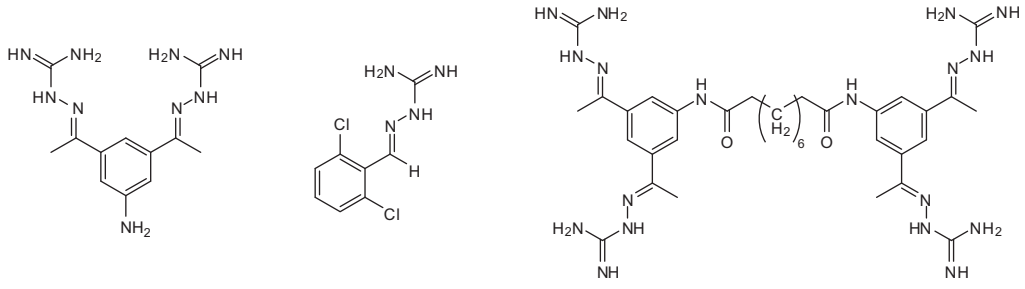
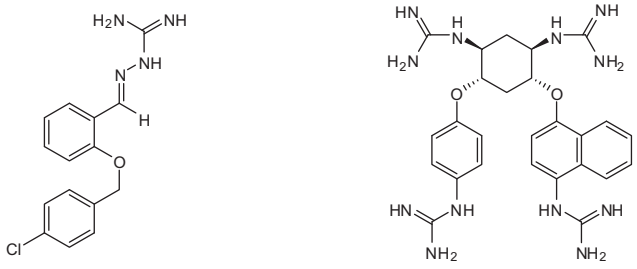


1 **guanabenz** **CNI-1493**



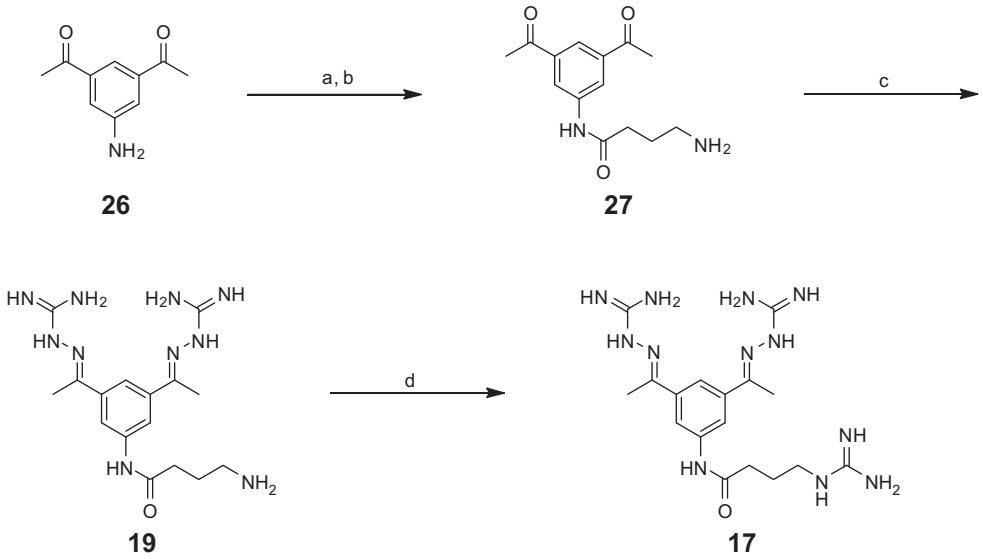
2 **compound 1e from Jiao et al.[6]**

its preference for substrates with basic P6-P1 residues.[19] Based on preliminary modelling we assumed that one amidinohydrazone group of **1** should occupy the S1 pocket, whereas the second one might bind into the S2 pocket. Therefore, we used the easily accessible aniline group of **1** for further modifications with basic residues to address additional acidic binding sites of furin (Table 2).

The arginine derivative **15** exhibits slightly enhanced affinity; a similar potency was found for its des-amino analogue **16**. Therefore, we also introduced the shorter 4-(guanidino)butyryl- and the homologous 6-(guanidino)caproyl residue (**17**–**18**). The most potent inhibitor **17** (preparation see Scheme 1) possesses a K_i value of 0.46 μM, which is approximately 4-fold improved compared to **1**. The γ-aminobutyric acid analogue **19**, an intermediate from synthesis of compound **17**, has slightly reduced inhibitory activity.

The most efficient non-peptidic furin inhibitor is a 2,5-dideoxystreptamine derivative with four guanidine residues (compound **1e** from Jiao et al.[6]), therefore, we prepared compound **20** by dimerization of **1** via a malonyl spacer to obtain a first analogue containing four amidinohydrazone groups (Table 3).

However, inhibitor **20** had only marginally improved potency compared to analogue **1**. Alternatively, we used chloroacetyl



26 **27** **19** **17**

Scheme 1. Reagents and conditions: (a) Boc-γ-aminobutyric acid, *N*-methylmorpholine, isobutylchloroformate, −15 °C, 10 min in DMF, followed by addition of **26**, 1 h at −15 °C and overnight at room temperature, (b) 1 N HCl in acetic acid, 1 h room temperature, (c) 2.6 equiv aminoguanidine hydrochloride, 5 mol % HCl, in 50% EtOH reflux for 6 h, (d) 3 equiv 1*H*-pyrazole-1-carboxamidine hydrochloride, 6 equiv diisopropylethylamine in DMF, 16 h. Final inhibitors **19** and **17** were purified by preparative reversed phase HPLC to a purity of >95% according to HPLC at 220 nm.