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New furin inhibitors based on weakly basic amidinohydrazones

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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

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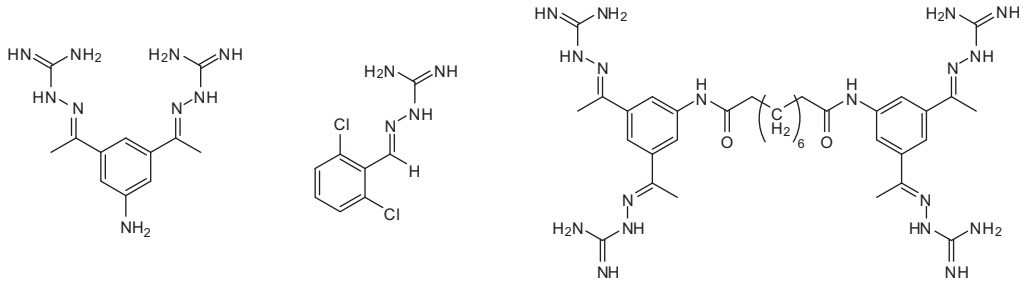
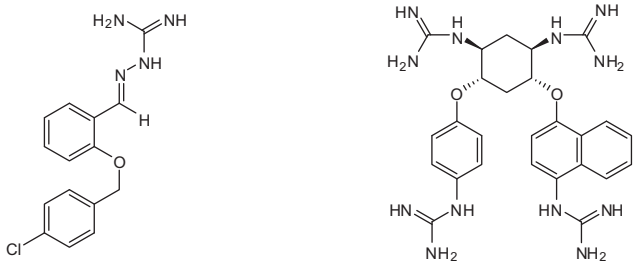


1 **guanabenz** **CNI-1493**



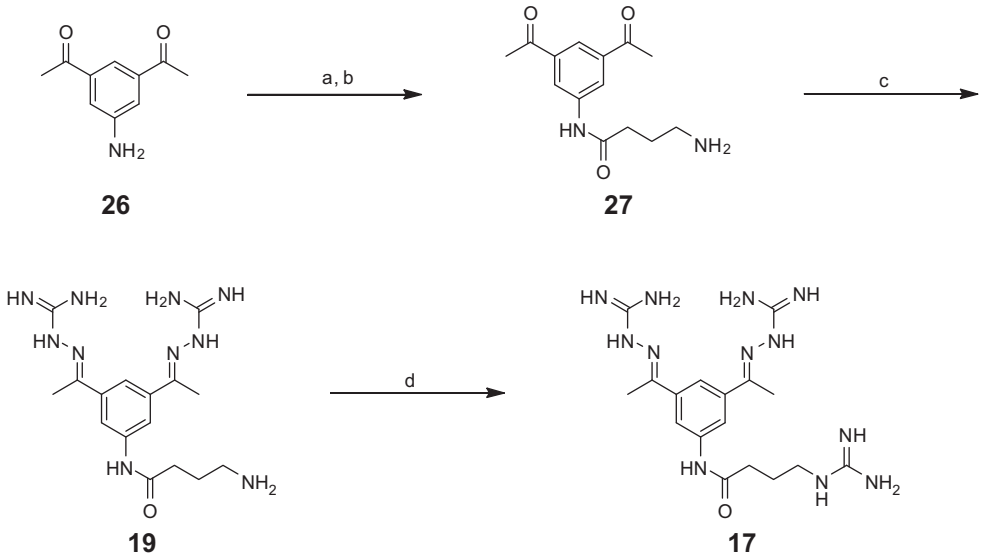
2 **compound 1e from Jiao et al.[6]**

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.

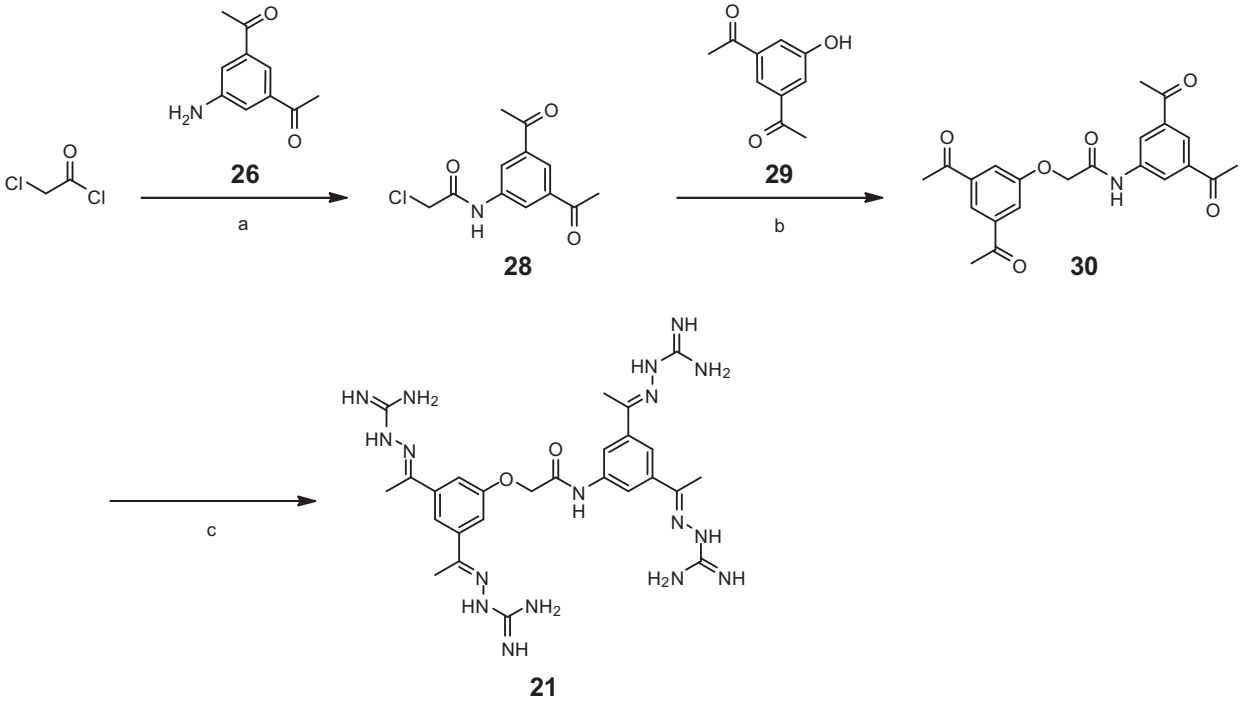
The most efficient non-peptidic furin inhibitor is a 2,5-dideoxystreptamine derivative with four guanidine residues (compound **1e** from Jiao et al.[6]), therefore, we prepared compound **20** by dimerization of **1** via a malonyl spacer to obtain a first analogue containing four amidinohydrazone groups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

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



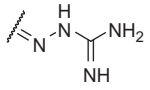
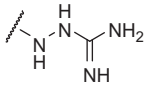
26 **27** **19** **17**

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.



Scheme 2. Reagents and conditions: (a) 1.2 equiv **26**, 1.2 equiv K_2CO_3 in dry dichloromethane, reflux 5 h, (b) 2 equiv **29**, 2 equiv Cs_2CO_3 in acetonitrile, reflux 5 h, (c) (i) 5.2 equiv aminoguanidine hydrochloride, 5 mol % HCl, in 50% EtOH reflux for 6 h; (ii) purification by preparative reversed phase HPLC.

Table 1
Amidinohydrazone and acylated aminoguanidine-derived furin inhibitors

R =  R^1 = 

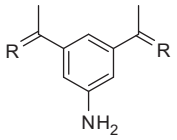
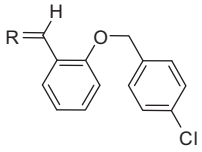
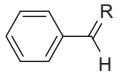
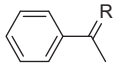
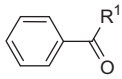
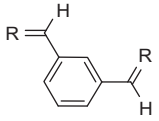
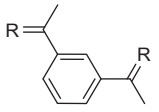
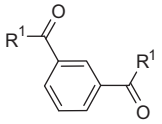
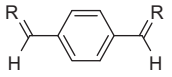
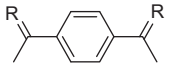
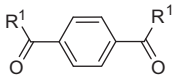
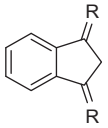
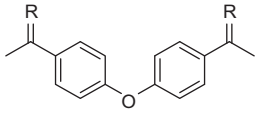
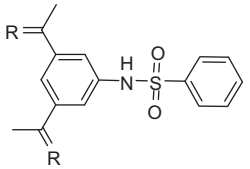
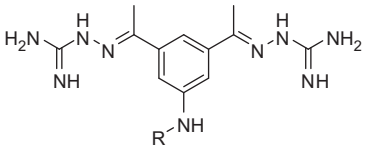
Compound	Structure	K_i (μM)[18]
1		1.82
2		25.3[a]
3		>500
4		273
5		376
6		11.5
7		4.78

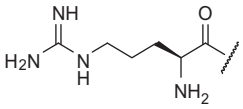
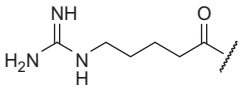
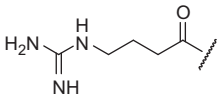
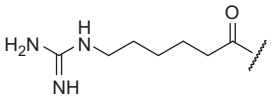
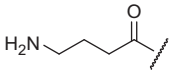
Table 1 (*continued*)

Compound	Structure	K_i (μM)[18]
8		27.9
9		1.47
10		1.84
11		173
12		17.3
13		44.0
14		1.55

[a] Komiyama et al. have reported a K_i value of 11.8 μM for this compound.[15]

Table 2
Furin inhibitors with the general formula



Compound	R	K_i (μM)[18]
15		1.13
16		1.04
17		0.46
18		1.42
19		1.2

chloride for the synthesis of inhibitor **21** (preparation see Scheme 2), which also allows access to bi- and trifunctional analogues **22–25**. Compound **21** has a slightly improved K_i value of 0.58 μM, which is similar to the potency of the guanidine derivative **17**, whereas the other analogues were less potent. The marginal differences in the inhibition constants of the compounds summarised in Table 3 suggest that amidinohydrazone groups from only one phenyl ring are able to make specific interactions with furin.

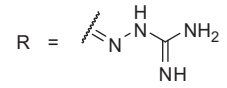
Selected compounds were also tested towards several trypsin like serine proteases, such as thrombin, trypsin, plasmin, factor Xa and subtilisin. Most K_i values were >10 μM; however, inhibitor **21** also inhibits thrombin (K_i = 0.86 μM), whereas compound **17** has a K_i value of 2.8 μM towards trypsin (see Supplementary data).

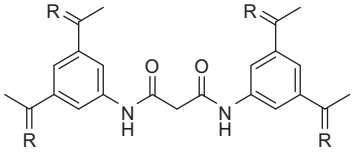
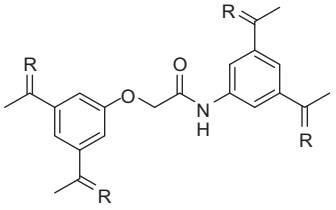
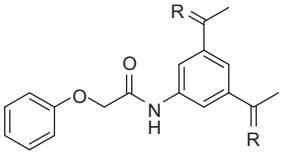
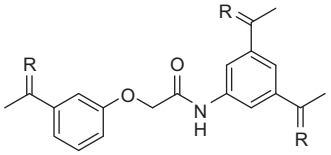
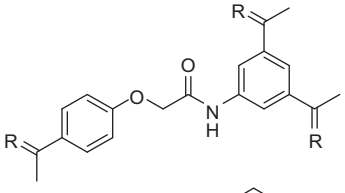
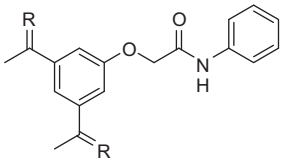
Although we could obtain only slightly improved inhibitors compared to our original screening hit, we assume that these amidinohydrazones might represent a suitable starting point for the design of non-peptidic furin inhibitors with relatively high selectivity against trypsin-like serine proteases. Despite several attempts, we failed to obtain a crystal structure of furin in complex with this type of inhibitors; therefore, we presently have no information regarding their binding mode. However, their reduced basicity may lead to improved pharmacokinetic properties compared to guanidine and amidine-derived furin inhibitors.

In general, all carbonyl compounds were converted to final inhibitors by treatment with aminoguanidine hydrochloride in ethanol and catalytic amounts of hydrochloric acid.[9] The synthesis of amidinohydrazone inhibitors is exemplarily described for inhibitors **17** and **19** (Scheme 1) and **21** (Scheme 2). Protected amino acids were coupled to intermediate **26**[9] using the mixed anhydride procedure, followed by removal of the protecting group and conversion to the amidinohydrazone (Scheme 1). Reaction of **19** with 1*H*-pyrazole-1-carboxamidine[20] provided inhibitor **17**.

Inhibitor **21** was prepared according to Scheme 2. Reaction of **26** with chloroacetyl chloride provided intermediate **28**, which was converted with the phenol derivative **29**[9] to compound **30**. Final conversion to the amidinohydrazones was performed as described in Scheme 1.

Table 3
Inhibition of furin by amidinohydrazone inhibitors



Compound	Structure	K_i (μM)[18]
20		1.13
21		0.58
22		5.61
23		1.46
24		1.94
25		1.35

Acylated compounds **5**, **8** and **11** were synthesized from reaction of corresponding acid chlorides with aminoguanidine × HCO_3^- in pyridine.[21]

Acknowledgements

The authors are grateful to Prof. Wolfram Bode and to Prof. Jörg Stürzebecher for initial contributions to this project.

Supplementary data

Supplementary data (a scheme with additional calculated pK_a values and a table with inhibition constants towards selected trypsin-like serine proteases are provided as Supplementary data) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.092.

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