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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.

Keywords

furin; proprotein convertase; protease inhibitor; amidinohydrazone; serine protease

Furin is a type-I transmembrane protein which contains a Ca^{2+}-dependent subtilisin-like serine protease domain. It is ubiquitously found in humans and the best characterized member of the family of proprotein convertases (PCs), which convert numerous precursors of secreted proteins to their active forms. Various studies revealed that furin plays a crucial role in many bacterial and viral diseases, tumorigenesis, neurodegenerative disorders or 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] also first potent nonpeptidic furin inhibitors have been described based on guanylated 2,5-dideoxystreptamines.[6] 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 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

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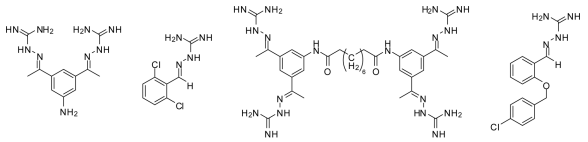
of 1.82 μM. This compound and several close analogues were originally described for the treatment of trypanosomiasis[9] and inflammation processes.[10]

Interestingly, there exists already 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]

Compared to other furin inhibitors, which often contain strongly basic guanidino or amidino groups, the fact that amidinohydrazones have a significantly decreased basicity might be advantageous; a pK_a of 8.1 has been reported for guanabenz[16], whereas guanidine has a pKa of approximately 13.



1 **Guanabenz** **CNI-1493** **2**

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 reference. For this compound we found a similar potency (K_i = 25 μM) as described in 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 meta and para position resulted in improved affinity, whereas both acylated aminoguanidines **8** and **11** were less active. Bis-amidinohydrazones **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 its preference for substrates with basic P6-P1 residues.[17] Based on preliminary modelling we assumed that one amidinohydrazone group of **1** should occupy the S1 site, whereas the second one might bind into the S2 pocket. Therefore, we used the simply accessible aniline group of **1** for further modifications with basic residues to address additional acidic binding pockets (Table 2).

The arginine derivative **15** has 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 activity.

The most efficient non-peptidic furin inhibitor described by Jiao (K_i = 6 nM) contains four guanidine residues,[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 marginal improved potency compared to analogue **1**. Alternatively, we used chloroacetyl-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 summarized in Table 3 suggest that only amidinohydrazone groups from one phenyl ring are able to make specific interactions with furin.

Selected compounds were tested also towards several trypsin like serine proteases, such as thrombin, trypsin, plasmin and FXa. For compound **21** we found an inhibition constant of 0.86 μM towards thrombin, whereas all other Ki values were > 10 μM.

Although we could obtain only slightly improved inhibitors compared to our original screening hit, we assume that such amidinohydrazones might be a suitable start point for the design of non-peptidic furin inhibitors with relatively high selectivity towards trypsin-like serine proteases. Despite several attempts we failed to obtain a crystal structure of furin in complex with these types 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.

Chemistry

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 pyrazol carboxamidine[18] 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.

Acylated compounds **5, 8** and **11** were synthesized from reaction of corresponding acid chlorides with aminoguanidine × HCO_3^- in pyridine.[19]

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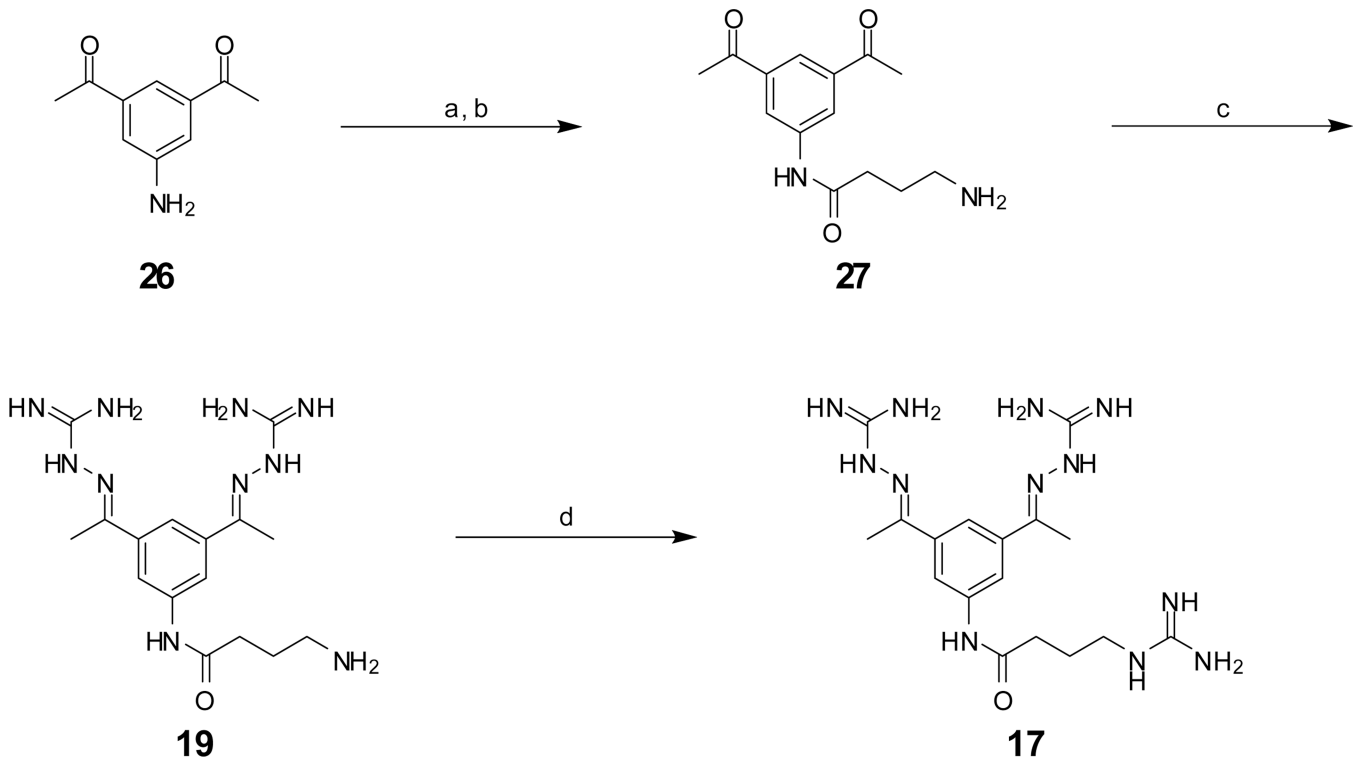
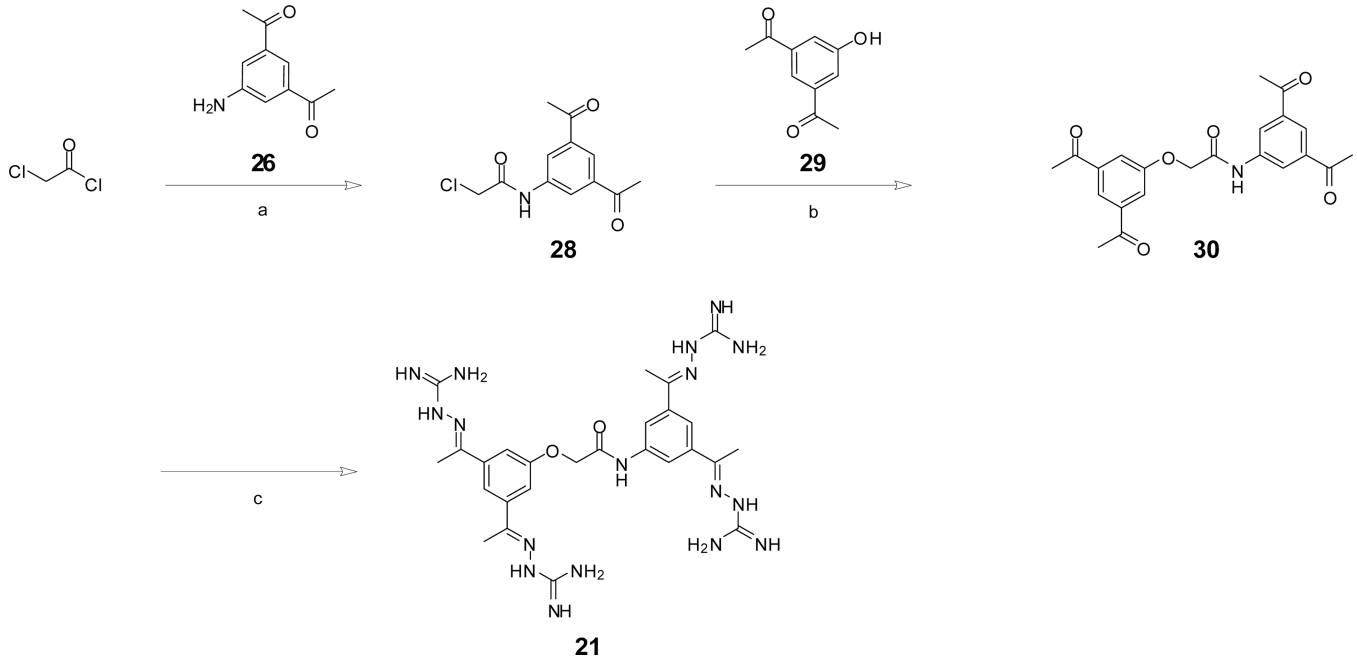


Scheme 1.

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

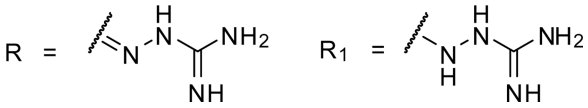


Scheme 2.

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

Table 1

Amidinohydrazone and acylated aminoguanidine-derived furin inhibitors



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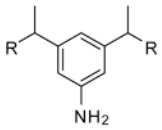
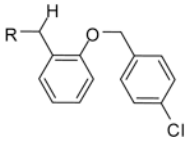
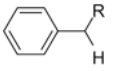
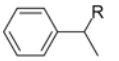
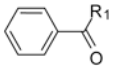
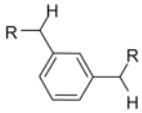
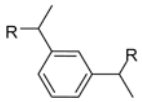
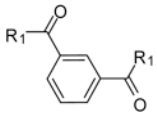
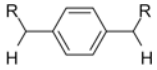
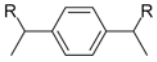
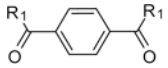
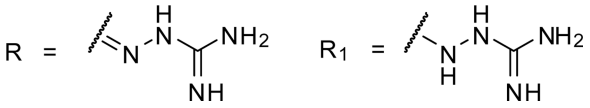
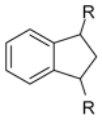
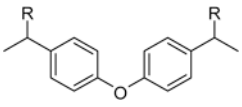
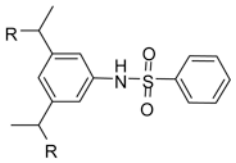
1		1.82
2		25.3#
3		>500
4		273
5		376
6		11.5
7		4.78
8		27.9
9		1.47
10		1.84
11		173



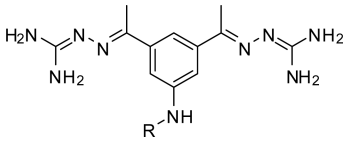
Compound	Structure	K_i (μM)*
12		17.3
13		44.0
14		1.55

*K_i values were obtained from Dixon plots as described previously[7]

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

Table 2

Furin inhibitors of the general formula:



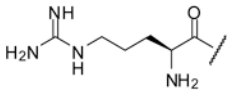
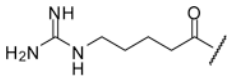
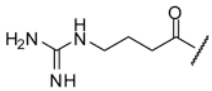
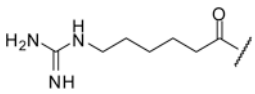
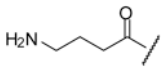
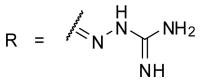
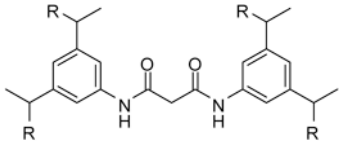
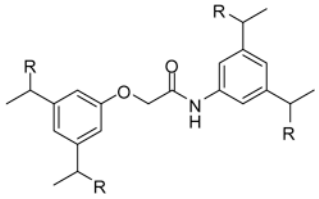
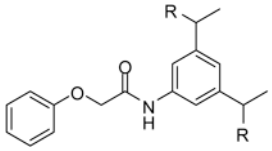
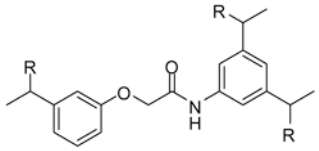
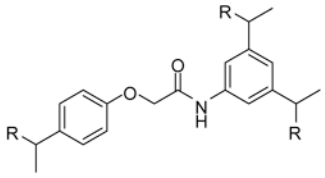
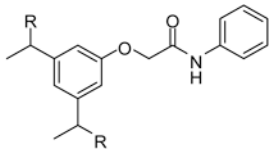
Compound	R	K_i (μM)
15		1.13
16		1.04
17		0.46
18		1.42
19		1.2

Table 3

Inhibition of furin by amidinohydrazone inhibitors



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