REVIEW

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CHEMICAL STRUCTURE AND PROPERTIES OF LOW-MOLECULAR FURIN INHIBITORS

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The review is devoted to the analysis of the relationship between a chemical structure and properties of low-molecular weight inhibitors of furin, the most studied proprotein convertase, which is involved in the development of some pathologies, such as oncologic diseases, viral and bacterial infections, etc. The latest data concerning the influence of peptides, pseudo-peptides, aromatic and heterocyclic compounds, some natural ones such as flavonoids, coumarins, and others on enzyme inactivation are considered. The power of furin inhibition is shown to rise with the increasing number of positively charged groups in the structure of these compounds. Peptidomimetics (Ki = 5-8 pM) are shown to be the most effective furin inhibitors. The synthesized substances, however, have not been used in practical application yet. Nowadays it is very important to find more selective inhibitors, improve their stability, bioavailability and safety for the human organism.

K e y w o r d s: furin, synthetic inhibitors, peptides, pseudo-peptides, derivatives of 2,5-dideoxystreptamine, enediynes, amidinohydrazones, flavonoids, coumarins.

Furin (EC 3.4.21.75), also known as PACE (Paired basic Amino acid Cleaving Enzyme) or SPCI (Subtilisin-like Proprotein Convertase I), belongs to the proprotein convertases (PCs) family, which is calcium-dependent serine endoproteinases involved in conversion of inactive precursors of proteins to their active forms [1-3]. PCs are involved in many important biological processes, such as embryogenesis and homeostasis. However, along with the normal physiological processes, proprotein convertases are implicated in the development of some pathologies, such as viral and bacterial infections, oncologic diseases, metastasis, obesity, diabetes, Alzheimer disease, etc. [1-3]. Therefore, these enzymes are considered to be promising targets for synthesis of inhibitors and development on their basis new therapeutic drugs [2, 4, 5].

Proteins and their fragments, natural components and low-molecular mass compounds with various structures have been studied in relation to their inhibitory effect on furin activity [6, 7]. Protein, peptide and pseudo-peptide inhibitors have some disadvantages, in particular, large size, toxicity (in some cases), instability, low cell permeability. Therefore, the search for potential furin inhibitors among non-peptide low-molecular weight compounds is a current issue of biochemical investigation, since such compounds are shown to have high metabolic and proteolytic stability [6, 8]. Furthermore, they are easily synthesized, and using their structural modifications enables analysis relationships between structure and activity.

It has been four years since our article [7] about proprotein convertase inhibitors was published. During this period a series of interesting research works describing new classes of substances for inactivation of these enzymes has been published.

The aim of this review was to analyze not only the latest research, but also some significant articles (published earlier) focused on structure and efficiency of low-molecular inhibitors of furin.

1. Furin as a member of proprotein convertase family and its role in biological processes

PCs constitute a family of proteolytic secretory enzymes, which are involved in processing of inactive protein precursors transforming them into "mature" forms through limited proteolysis of the polypeptide chain at one or several inner sites. PCs group includes nine proteinases: seven of which, namely, furin, PC1/3, PC2, PC4, PACE4, PC5/6 and PC7 are specific for positively charged basic amino acids, and two – for non-polar amino acid residues [2, 3]. Under their action proproteins are transformed into biologically active peptide hormones, receptors, growth factors, neuropeptides, regulators of cholesterol metabolism, and enzymes involved in growth maintaining, metabolism and other normal physiological process. PCs were shown to play an essential role not only in normal homeostasis, but also in development of various pathological processes [1-3].

One of the most studied enzymes of PCs family is furin, which was found in almost all studied human tissue and in all studied cell types. Furin expression and activity are required for development of such pathologies as cell transformation and tumor progression, metastasis and angiogenesis [1, 9, 10]. Furin is also involved in development of Alzheimer's disease [11], non-Alzheimer brain amyloidosis [12], osteoarthritis [13], and atherosclerosis [14]. Furthermore, bacterial toxins such as anthrax toxin [15], exotoxin *Pseudomonas* A, diphtheria toxin [16], *Shiga* toxin [17], dermonecrotic toxin *Bordetella* [18] are only activated after their cleavage by furin or other PCs of respective precursors. Furin plays an essential role in the spread of viral infections such as avian influenza (bird flu) [19], hepatitis B [20, 21] and human immunodeficiency [22].

Furin is synthesized as a pre-proprotein, which contains: a signal peptide, a prodomain, subtilisin-like catalytic domain, middle P-domain, site enriched with cysteine residues, transmembrane "anchor" and cytoplasmic "tail". N-terminal prodomain, which consists of 81 amino acis residues (Gln27-Arg107), acts as a peculiar inhibitor [23, 24]. Profurin (104 kDa) becomes a proteolytic enzyme (98 kDa) after autocatalytic cleavage of its prodomain [25, 26]. It is accumulated in the trans-Golgi network (TGN) and circulated between the TGN, cell surface and endosomes, activating on this pathway large number of the target proteins [1]. The enzyme active site contains (similar to other serine enzymes) triad of amino acids Ser, His and Asp, as well as Asn residue. Furin belongs to Ca^{2+}-dependent type I transmembrane proteins. The enzyme identifies motif, enriched for basic amino acid cluster on the substrates, and enhances hydrolysis of the peptide chain, which contains sequences Lys-Arg or Arg-Arg at the C-terminal [27]. It occurs, because substrate-binding site of the enzyme is shaped as a narrow canyon, rich in negatively charged Glu and Asp residues. It was shown by Shiryaev et al [28] that the presence of solely R-X-R /K/X-R motif in the substrate structure is insufficient for the realization of narrow furin specificity, since both short-range P_4-P_1 as well as long-range P_7-P_6 interactions of the enzyme are important.

Blocking of the enzyme activity leads to reducing or complete termination of the pathological effects mentioned above, and it might become a promising treatment therapy. Therefore, inhibition of furin activity should be considered as a potential therapeutic strategy for the treatment of cancer, viral and bacterial infections, especially in short-term therapy [4].

To date, various furin inhibitors based on proteins, peptides and pseudo-peptides have been developed [6, 7]. However, high molecular mass of proteins, lack of selectivity, low cell permeability impede wide use of these compounds as pharmaceutical agents, although the search for furin inhibitors among both natural and recombinant proteins still continues.

For instance, in 2012 Zhu et al. [29] applied, dromedary VHH antibodies (so-called nanobodies), derived from dromedary heavy chain immunoglobulin, against catalytically active enzyme as specific furin inhibitor. These antibodies bound only to furin and not to other PCs. They inhibited cleavage of proteins, but did not block hydrolysis of low-molecular mass peptide substrates. This indicates that the mechanism for nanobodies action based on steric hindrance. Nanobodies were the first generation of non-competitive and specific furin inhibitors [29].

It is important to search selective low-molecular mass non-peptide furin inhibitors, which could be proteolytically more stable and available by synthetic methods [8].

In the early 2000's, polyarginines were found to inhibit furin [30, 31]. Ramos-Molina et al [32] demonstrated that cyclic polyarginines, used as transduction reagents and containing tryptophan residues, were also able to inhibit intracellular proprotein

convertases activity. In particular, cyclic decapeptides containing alternating tryptophan and arginine residues in its ring (cyclo-[WRWRWRWRWC] or cyclo-[WRWRWRWRWR]), as well as cyclic hexapeptides, which contain a hydrophobic "tail" N-acetyltetratryptophan (Ac-WWWW-cyclo[KRRRRR]) or dodecanoic acid residue (dodecanoyl-cyclo[KRRRRR]) outside the ring system were found to exhibit significant inhibitory effect on the furin activity both *in vitro* (K_i = 0.1-1.0 μM) and within cells. Importantly, these cyclopeptides did not exert any toxic effect on cells.

Steinmetzer T. et al. made a great contribution to the development of highly potent furin inhibitors [33-36]. They synthesized substrate-like peptides and peptidomimetics containing 4-amidino-benzylamide (4-amba) group at the molecule C-terminal and acyl residues with basic groups, for example, guanidine or amino groups at the N-terminal of sub-center P_5. These compounds contained both natural and non-natural amino acid residues at P_3. To date, the most potent non-covalent furin inhibitor is peptidomimetic **1** (K_i = 5.5 nM), which contains non-natural amino acid tert-leucine (Tle) residue at sub-center P_3 (Fig. 1). Peptide **2** with valine at P_3 sub-center exhibits reduced affinity for furin (K_i = 8 pM).

The synthesized compounds were found to effectively inhibit other PCs such as PC1/3, PC4, PACE 4 and PC5/6 (K_i = 1.7-810 nM), and in model experiments they exhibited high activity against anthrax and diphtheria toxins [35, 36].

2. Small molecule carbocyclic furin inhibitors

Among non-peptide low-molecular mass inhibitors of furin, diterpene derivatives should be noted [37], which were found in *Andrographis paniculata* plant, widely spread in Southeast Asia and India. The main components of this plant are andrographolide (**3**) and its derivatives (Fig. 2).

It was shown that these compounds exhibited a low inhibitory activity toward furin, PC1 and PC7. IC_{50} values for the studied derivatives ranged from micromolar to millimolar concentrations. Among the studied compounds, neoandrographolide **9** was found to be the most potent furin inhibitor (IC_{50} = 53.5 μM), that is nearly 20-fold more effective than andrographolide itself **3** (IC_{50} = 1.0 mM, and K_i = 200 μM). Such difference might be due to the unique substitution by O-glycoside residue at the third carbon atom. This indicates that the specific chemical modification of the initial compound might lead to a molecule with higher capability to inactivate furin. This observation was supported by data obtained for succinic acid esters resulted from esterification of hydroxyl group at the C3 atom.

Jiao G.-S. et al. investigated 2.5-dideoxystreptamine derivatives as small molecule inhibitors of furin [38]. Data as to effectiveness of these compounds to block furin are shown in Table 1.

It was shown that the most potent furin inhibitor was 2.5-dideoxystreptamine derivative with 4 guanidine substituents (compound **14**). It was revealed that with increasing of substituent R_1 size (compounds **15** and **24** vs. 11), affinity for furin decreased. Derivatives containing two aryl substituents were found to be slightly more effective inhibitors than those containing more aryl substituents (compound **16** vs. **23**). Introduction of guanidine group into para-position also led to better results (compound **16** vs. **17**). The nature of linker, which binds the aromatic moiety and 2.5-dideoxystreptamine, also impacted the blocking capability. It was evidenced by a significant decrease in the inhibitory activity when oxygen atom of the ester group was substituted by a carbamate group (-NHCOO-) (compounds **25-27**).

It was found that 2.5-dideoxystreptamine derivatives are competitive inhibitors of furin with K_i values at nanomolar range that is comparable to the potency of protein- or peptide-like inhibitors. In addition, these compounds exert certain selectivity towards furin and PC5/6, therefore they can be used in therapy as anti-bacterial and anti-viral agents.

Analysis of the structure-activity relations in these compounds revealed there is no linear correlation between the inhibitory activity and amount of positively charged guanidine groups in their struc-

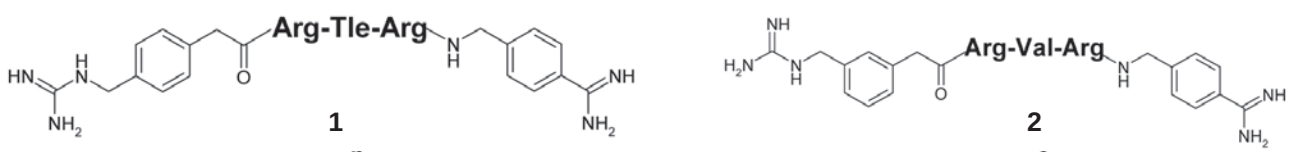


Fig. 1. Chemical structure of pseudopeptides – the most effective furin inhibitors [35, 36]

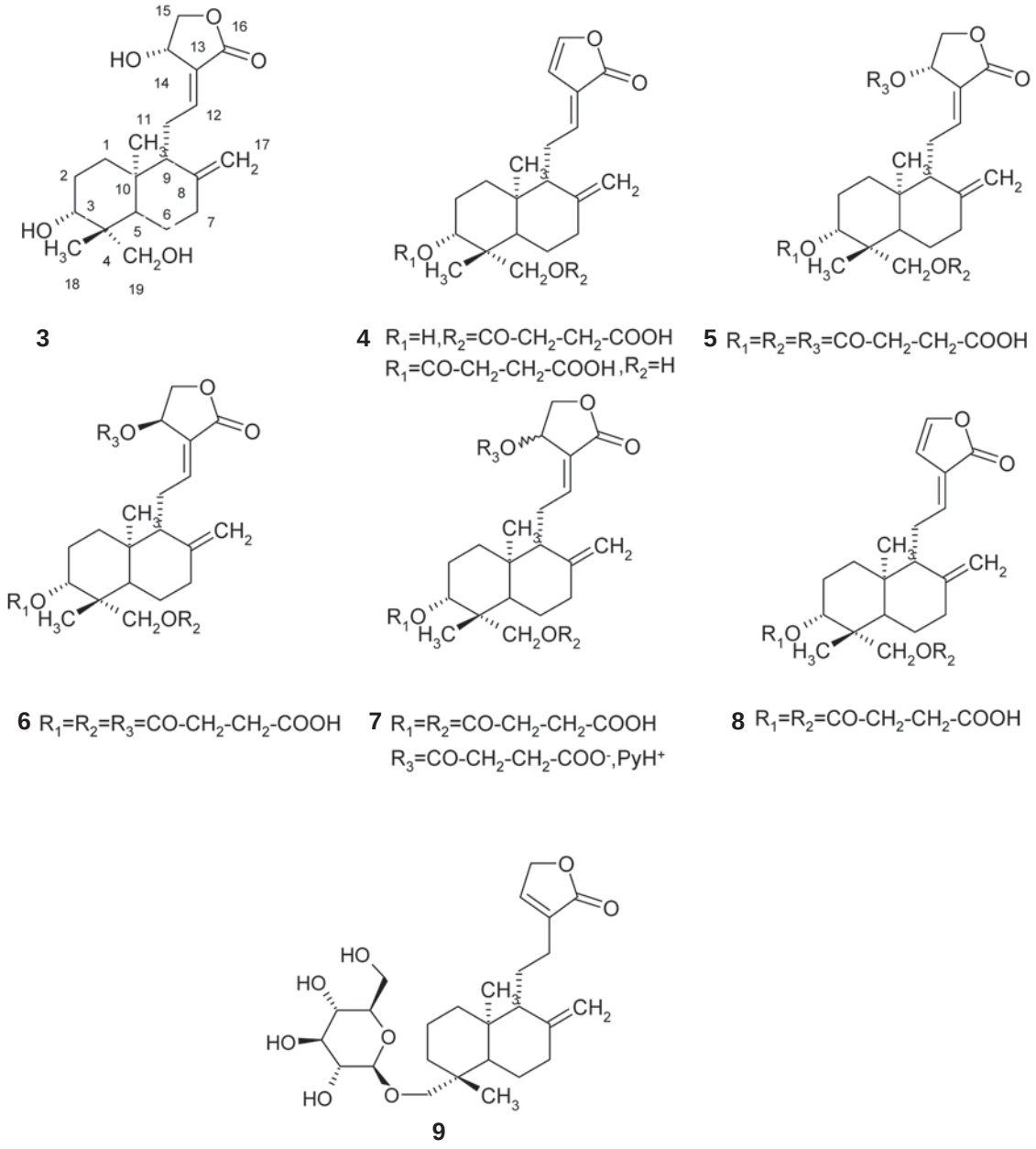
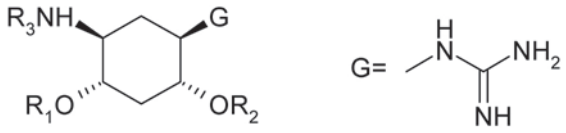


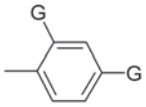
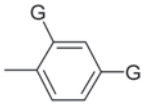
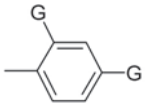
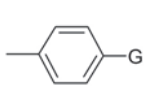
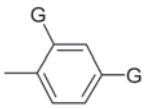
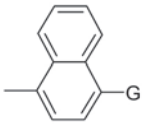
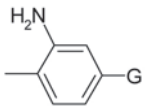
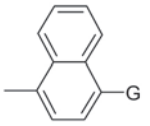
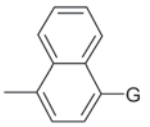
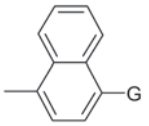
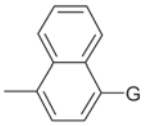
Fig. 2. Chemical structures of non-peptide andrographolide-based furin inhibitors: ***3*** *– andrographolide;* ***4*** *– 14-dehydroandrografolide succinic acid monoester (DASM);* ***5*** *– succinic ester of andrographolide-1 (SEA-1);* ***6*** *– SEA-2;* ***7*** *– SEA-4;* ***8*** *– SEA-5;* ***9*** *– neoandrographolide [37]*

tures. These findings are not consistent with data on inhibitory activity of polyarginine peptides, for which the correlation between inhibitory efficiency and the number of positive charges in a molecule was established [39]. The differences in results could be explained by peculiarities of 3-dementional guanidine groups' arrangement relative to the aromatic ring and by nature of the linker between the 2.5-dideoxystreptamine core and aryl groups

In 2015 Ramos-Molina et al [40] studied dependence of the anti-firin activity on structures of 2.5-dideoxystreptamine derivatives (Table 1) and bis-guanidinophenyl esters derivatives characterized by linkers with different hydrophobicity and containing two or four guanidine groups (Fig. 3, compounds **28-33**). The authors assessed their activities directly in cells using the following methods: a) enzyme activity assay in the TGN (trans-Golgi

Table 1. Inhibition constants (K_i) for 2.5-dideoxystreptamine derivatives [38]



Compound	R_1	R_2	R_3	K_i, μM
10				0.169±0.009
11				0.089±0.022
12				0.404±0.018
13				0.022±0.002
14				0.006±0.002
15				0.069±0.004
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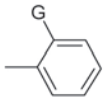
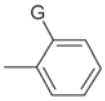
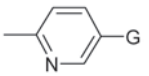
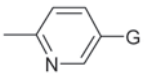
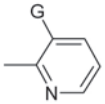
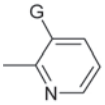
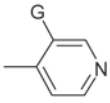
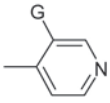
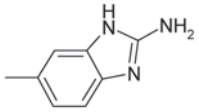
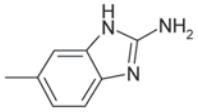
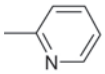
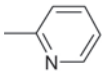
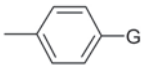
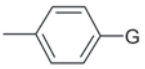
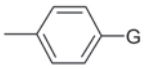
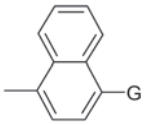
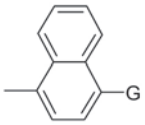
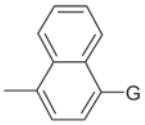
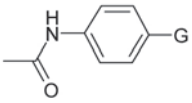
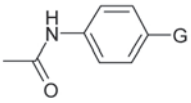
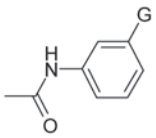
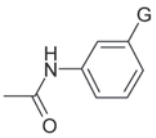
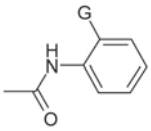
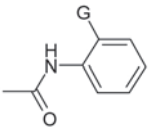
17				>100
18				0.042±0.003
19				>100
20				>100

Table 1. Continuation

21				>100
22				>100
23				0.046±0.003
24				0.423±0.005
25				0.812±0.041
26				1.768±0.007
27				>100

network) using created chimeric substrate CHO-GRAPfurin; b) testing of HT-1080 fibrosarcoma cells migration to assess furin processing of matrix metalloproteinases (in the TGN), c) measurement of *Pseudonomas* exotoxin A cytotoxicity on CHO-DG44cells to assess the furin activity in the endocytic pathway; d) testing of anthrax cytotoxicity on macrophage RAW 264.7 cells to examine the furin activity on the cell surface. Additionally, the effect of the synthesized compounds on cell survival was studied. The tested compounds were divided into four groups. In the first group (Table 1, compounds **10**, **11**, **16**, **17**), effect of amount of guanidine groups and their relative position in the molecule on furin was studied. In the second group of inhibitors (Table 1, compounds **18-20**, **22**), effect of the presence of heteroatoms (such as nitrogen) in benzene ring of 2,5-dideoxystreptamine derivatives was assessed. In the third group of compounds (Table 1, compounds **12**, **14**, **15**), effect of hydrophobicity of the mentioned derivatives was investigated. Finally, in the fourth group (Table 1, compounds **25-27**), effect of guanidine group positions relative to the linker in the *ortho*-, *meta*- or *para*-positions was analyzed. The bis-guanidinophenyl esters structures, which demonstrate the effect of nature of the linker on anti furin activity of the compounds **28-33** are shown in Fig. 3.

The performed comprehensive study emphasized the importance of synthesis of compartment-specific furin inhibitors, and found that the best correlation between the K_i values determined *in vitro* and anti furin activity determined inside cells was observed for furin functioning on the cell surface. Authors believed that they succeeded to identify the compounds, which possess a wide spectrum of biological activities without marked toxicity.

3. Pyrrolidine, guanidines and terpyridines as effective PCs inhibitors

Among the nitrogen-containing small-molecule furin inhibitors, 2,3,5,6-tetrahydro-1*H*-imidazo[1,2-a]imidazole derivatives were studied [41].

These compounds can be referred to aromatic bicyclic guanidines. They exhibited inhibitory activity toward furin with K_i > 15 μM, whereas their

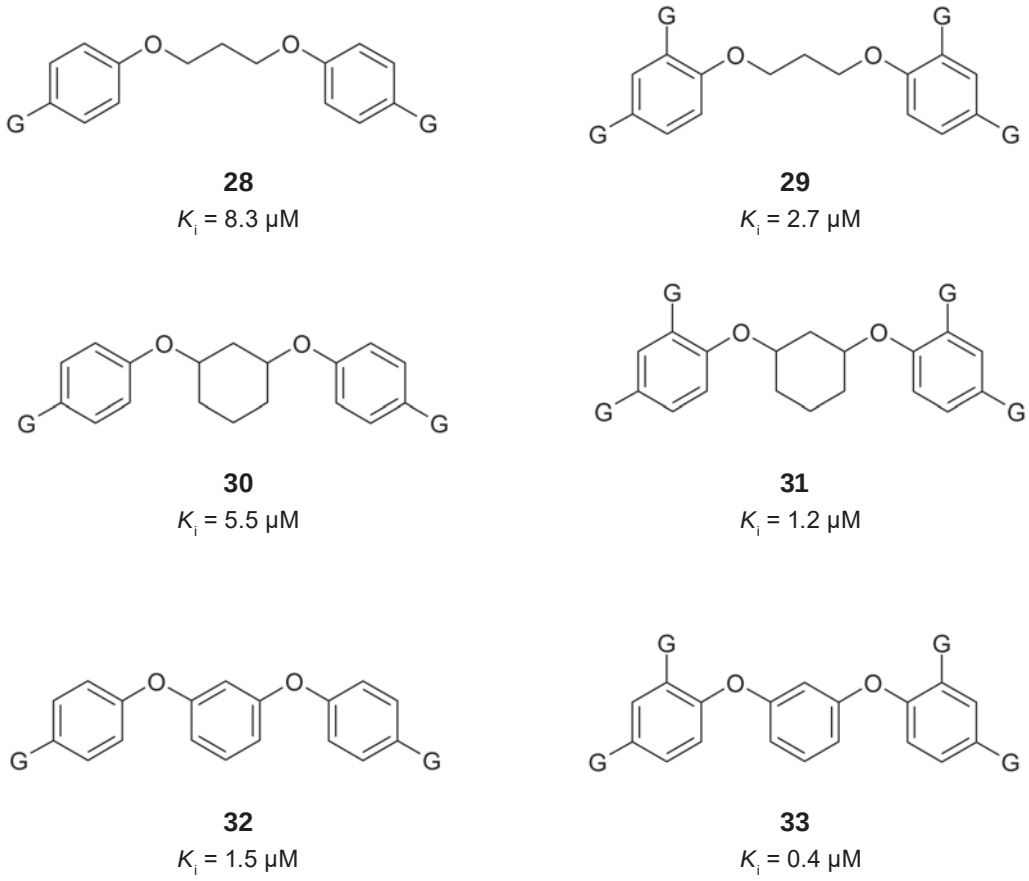


Fig. 3. Structure and activity of bis-guanidinophenyl esters (G - guanidine group) [40]

inhibitory activity toward protein convertase PC2 was much lower – 3.3-10.0 μM. Among the studied compounds based on pyrrolidinebispiperazine, inhibitory activity toward furin was found to be higher than 25 μM that is not significant result. But these substances showed remarkable results with the PC2 inactivation (K_i = 0.54-0.66 μM) and were found to be first established selective inhibitors of this enzyme. To obtain potential furin inhibitors on the basis of these compounds, further researches of their structural modifications are required.

Podsiadlo P. et al. [42] showed that some stable complexes of zinc and copper can effectively block furin (Table 2). Structures of terpyridine derivatives that are constituents of chelate complexes are presented in Fig. 5. Free chelating molecules did not inactivate the enzyme. Compounds, type of Cu(TTP)Cl_2 and Zn(TTP)Cl_2, blocked furin activity with the IC_{50} values ranged from 5 to 10 μM. Free Zn^{2+}-ions exhibited less inactivating effect than copper complex compounds. Although, Cu^{2+}-ions were more effective than chelate complex Cu(TTP)Cl_2. In general, Cu-ions had a greater ability to bind to the enzyme active site owing to their coordination with catalytic residues. Inhibition was competitive and irreversible. The effectiveness of inhibition depended on the nature of the substituent in chelate complex. These substances, in general, are stable and promising for research on bioterrorist protection.

4. Non-peptide heterocyclic and aromatic enediynes

Basak et al. [43] presented an innovative strategy for the synthesis of effective PCs inhibitors on the basis of aromatic and heterocyclic derivatives (so-called enediynes). The approach was based on incorporation of enediynyl amino acid (Eda) into a polypeptide chain QQVAKRRTKR↓DVYQE at the P_1-P_1'-site (marked with arrow), which correspond to amino acid 98-112 sequence of human furin prodomain [43]. The Bergman cycloaromatization reaction [44] led to a free radical generation resulting in a sufficiently high reactivity of these derivatives in the enzyme inactivation. Insertion of Eda also contributed to the formation of polypeptide chain β-turns. As a result, a potent furin inhibitor (IC_{50} ~ 40 nM)

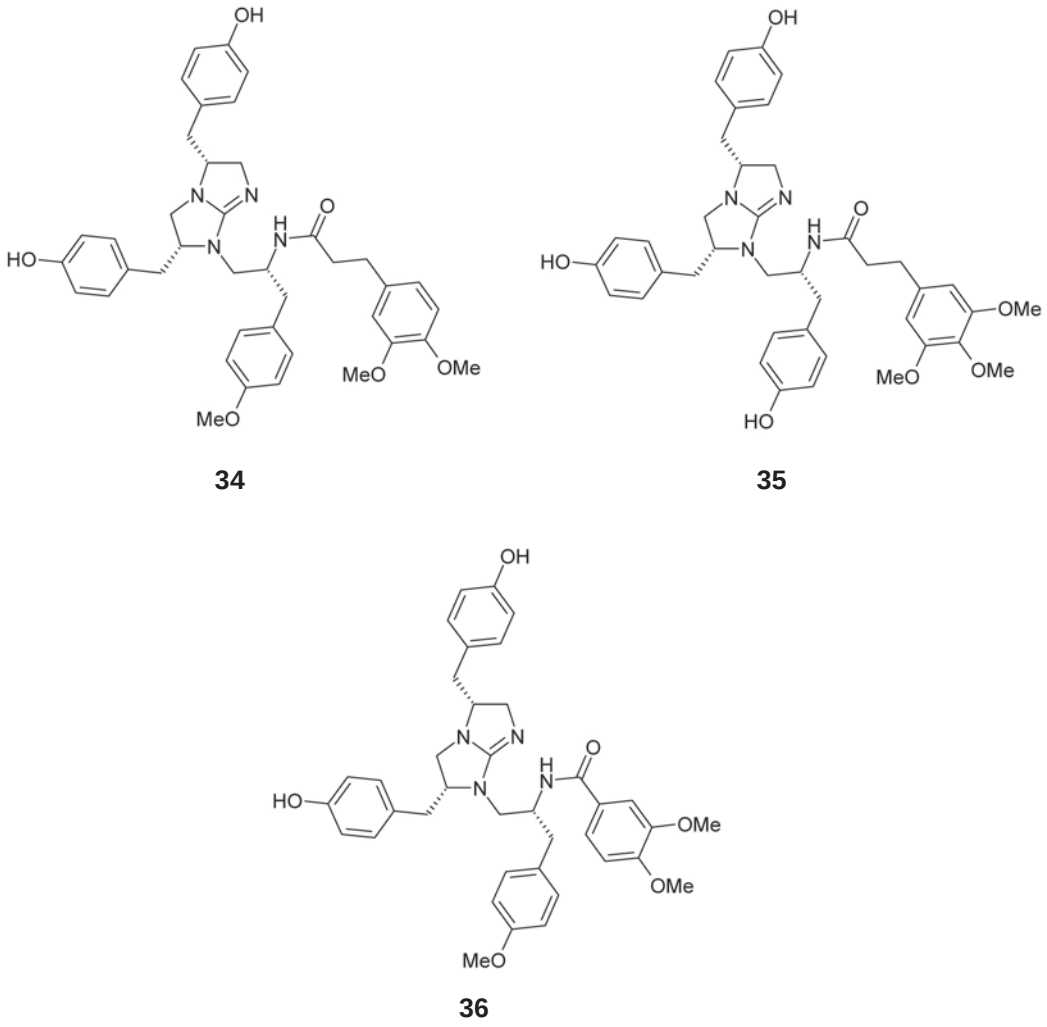


Fig. 4. Aromatic bicyclic guanidine derivatives that inactivate furin [41]

Table 2. Effectiveness of zinc and copper compounds in furin inhibition. The mixtures were incubated for 2 h at 22 °C prior to the substrate addition. Buffer: 20 mM NaMES, pH 7.0, 0.1% Triton X-100, 3% DMSO, 20 mM [NaCl], 1 mM [$CaCl_2$] [42]

N	Compound	IC_{50}, μM	Compound	IC_{50}, μM
37	Zn^{2+}ion	21.0 ± 3.0	Cu^{2+} ion	0.14 ± 0.06
38	Zn(MPT)Cl_2	9.0 ± 1.2	Cu(MPT)Cl_2	5.1 ± 0.6
39	Zn(TTP)Cl_2	9.0 ± 1.1	Cu(TTP)Cl_2	5.0 ± 0.6
40	Zn(Me_2-4′-TTP)Cl_2	14.0 ± 2.0	Cu(Me_2-4′-TTP)Cl_2	14.0 ± 1.5
41	Zn(TERPY)Cl_2	-	Cu(TERPY)Cl_2	7.7 ± 0.5
42	Zn(*t*-Bu_3-TERPY)Cl_2	No inhibition	[Cu(TERPY)Cl](OCl_4)	6.9 ± 0.5
43	Zn(Cl-TERPY)Cl_2	70.0 ± 7.0	Cu(OH-TERPY)Cl_2	7.2 ± 0.7

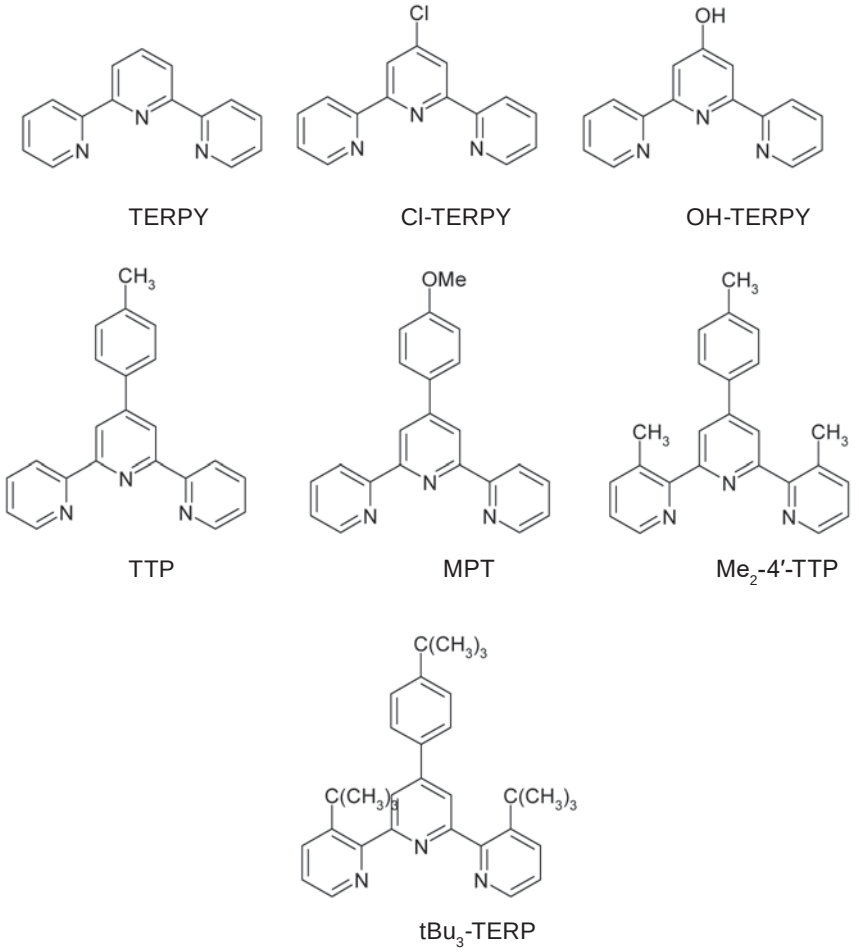


Fig. 5. Structures of terpyridine derivatives, which inhibit furin activity [42]

was obtained upon cleavage of synthetic fluorogenic substrate Boc-RVRR-MCA or FRET-derived protein hSARS-CoV (IC_{50} ~ 193 nM). Eda-peptide 98-112 also effectively blocked processing of precursors of such growth factors as PDGF A, B and VEGF-C in CHO cells.

In the work [43] it was shown for the first time that various aromatic enediynyl derivatives exhibited inactivating effect on furin as well as on PC5 or PC7 activities with IC_{50} values ranging from 8.5 to 193 μM depending on the enzyme nature and structure of enediynyl derivatives. The most effective inhibitor was found to be a compound composed of 12-membered heterocycle with bis-1,4 nitrogen atoms and two benzene side chains (Fig. 6, compound **44**).

44
IC_{50} = 10.5 ± 1.8 μM

45
IC_{50} = 14.2 ± 1.8 μM

Fig. 6. Structures of aromatic enediynes and IC_{50} values for furin inactivation [43]

5. Natural compounds containing oxygen

5.1. Flavonoids

Among the oxygen-containing furin inhibitors, natural flavonoids, such as baicalein, chrysin, oroxylin A and its glycoside were studied (Fig. 7, compounds **46**-**49**) [45]. These derivatives are the main components of *Oroxylum indicum* plant. It was previously found that baicalein and its glycoside (7-D-β-glycoside), known as baicalin, the major component of another medicinal plant *Scutellaria baicalensis*, effectively blocked cancer cell growth *in vitro* [46]. Authors hypothesized that anti-cancer properties of flavonoids related to their ability to inhibit PCs activity. Clear correlation between furin inactivation

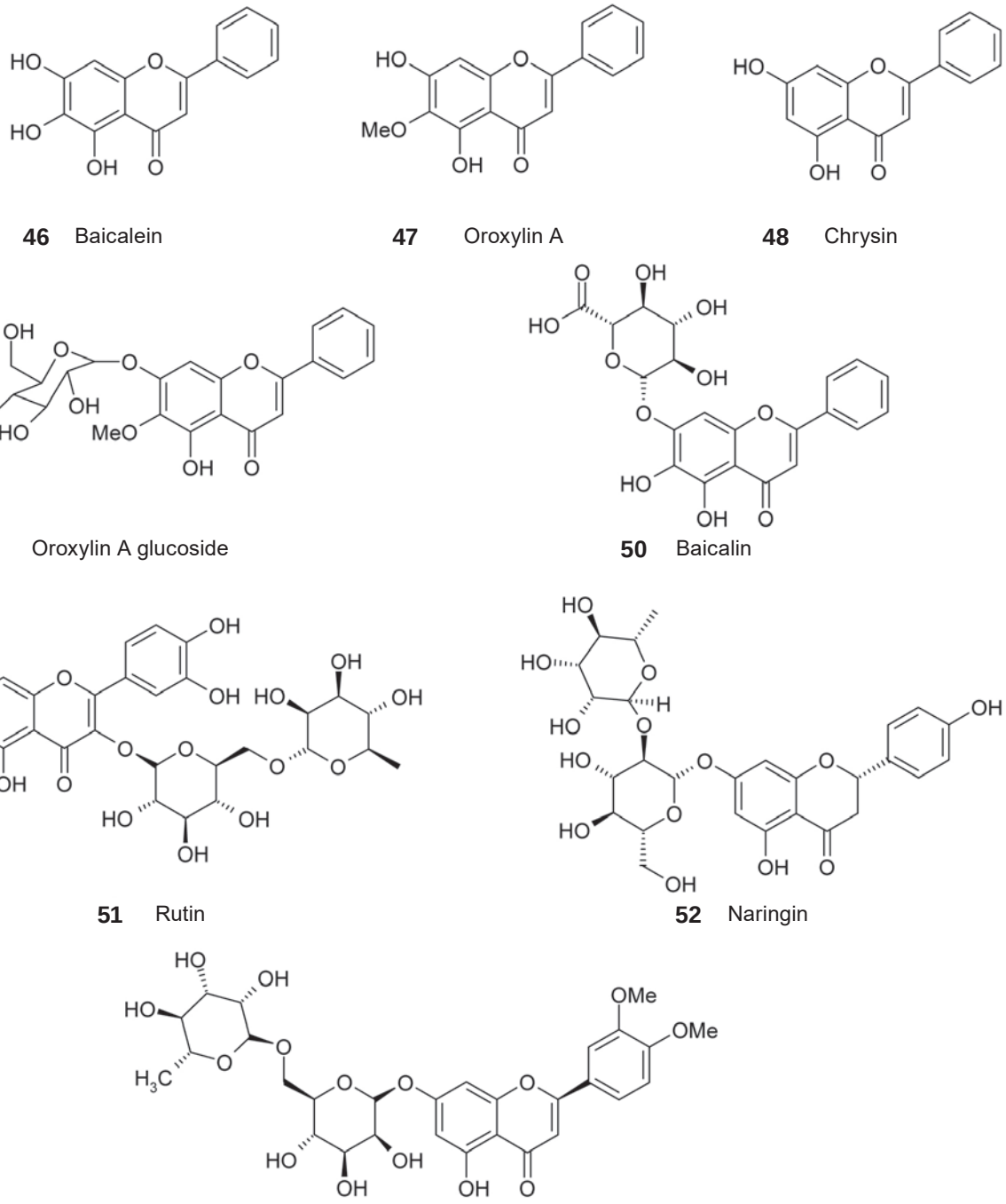


Fig. 7. Flavonoids, which block the furin and other PCs activities

and regression of cancer cells CT-26 (colorectal carcinoma), widely used in such studies, was observed. Data on cancer cell proliferation and migration revealed that baicalein and oroxylin A glycoside were more effective (among tested flavonoids) in suppressing of growth and migration of pathogenic cells. It was also found that baicalein (compound **46**), which did not have the carbohydrate moiety in its structure, inhibited more effectively growth and proliferation of cancer cells than its glycoside – baicalin (compound **50**).

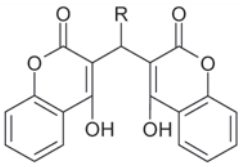
Majumbar et al. [45] demonstrated that these flavonoids exhibit inhibitory effect against furin and other PCs. K_i and IC_{50} values were found to be in the range of 5-35 μM. Thus, oroxylin A was the most potent furin inhibitor with K_i = 5.0 μM that is 5- and 7-fold higher than for baicalein and chrysin, respectively. Its anticancer effectiveness was lower than that of baicalein and oroxylin A glycoside. This difference might result from the involvement, besides furin, other PCs (particularly PCSK6) in the process of tumor growth. A comparative analysis of the inhibitory effectiveness against furin, PCSK4, PCSK5 and PCSK7 revealed that only baicalein has selectivity for PCSK4.

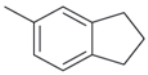
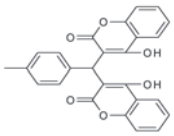
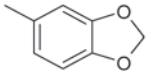
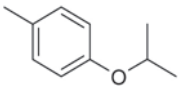
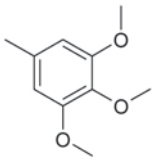
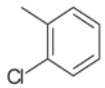
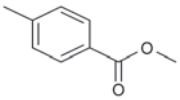
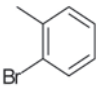
Previously, it was found that other members of the flavonoid family, namely baicalin, rutin, naringin and methyl hesperidin (Fig. 7, compounds **50-53**) also inhibited activity of furin with K_i values ranged from 80 to 200 μM. Since rutin inhibited the furin activity via reversible competitive mechanism [47], we can assume that this is mediated by direct binding of the inhibitor molecule to the enzyme active site. The interaction can occur owing to hydrogen bonds between hydroxyl groups of glycosylated fragment and amino acid residues of the enzyme active site. This finding is consistent with the results on inhibition of furin by andrographolide 3 and glycosylated derivative 9. The effectiveness of inhibition depends on 3-D-dementional position of the glycoside residue that could provide efficient interaction with furin binding center.

5.2. Furin inactivation by components of natural products: coumarins, quinonoids and iridoids

Among the small molecule inhibitors of furin, derivatives **54-62** of dicoumarol, which is used in clinic as an anticoagulant and is notable for its high bioavailability, low toxicity and high cell permeability, were studied [48] (Table 3).

Table 3. Furin inhibitors based on dicoumarol [48]



Compound	R-	K_i, μM
54		1.04
55		3.3
56		6.05
57		18.8
58		20.8
59		22.0
60		78.3
61		145.2
62		185.1

These compounds were selected after thorough screening of their furin inhibitory effect given K_i values from 1.04 (compound **54**) to 185 μM (compound **62**). 4-Hydroxycoumarin, warfarin and 3-(α-acetonyl-phenyl)-4-hydroxycoumarin, which can be considered as structural units of dicoumarol derivative molecules, were found to be capable of inhibiting the enzyme only in millimolar concentrations. That is, the unique dicoumarol structure is the cause for their more effective furin inhibition.

Furin inactivation can occur both at the cell surface (in case of anthrax toxin) and in the secretory pathways (blocking of metastasis growth factor - membrane type-1 matrix metalloproteinase (MT1-MMP)) at the micromolar concentrations, and mechanism for the inhibition was shown to be non-competitive. Inhibition of the enzyme activity by compounds **56**, **57** and **62** were reversible, indicating the lack of non-specific reactions between these derivatives and the enzyme. However, selectivity for furin was not observed, since these compounds also exhibited inhibitory effect on rat PC PACE4 and human PC5/6 and PC7.

K_i and IC_{50} values for furin inhibition on the cell surface as well as for intracellular processing were found to be in the low micromolar range. These values significantly differed from those for furin inhibitors of peptide and protein structure, which were ~ 1000–10 000 K_i. For comparison, IC_{50} values for blocking of the intracellular processing by compounds **54**, **55** and **57** were ~ 4 K_i, 17 K_i, and 1 K_i, respectively. This highly effective inhibition can be associated with dicoumarol ability to block both intracellular enzyme activity and enzyme activity on the cell surface.

Dicoumarols are used in medicine as anticoagulants of indirect action, which makes them an attractive platform for structural modifications. Structural optimization of dicoumarol-based inhibitors is required to achieve selectivity for furin and other PCs. The search for more effective inhibitors will give an opportunity to investigate thoroughly dicoumarol binding sites, as well as the mechanism for non-competitive inactivation of furin.

Among simple coumarins, just 4-hydroxycoumarin, 4-hydroxy-3-(3-oxo-1-phenylbutyl)coumarin and 4-hydroxy-3-(3-oxo-1-phenylpropyl)coumarin inhibited furin activity (Fig. 8, compounds **63-65**) [8]. The most effective inhibitor *in vitro* was scopoletin (or MCD-1) 66, isolated from a medicinal plant *Morinda citrifolia* (Table 4) [8]. This compound inhibited the enzyme using a fluorogenic substrate Boc-RVRR-MCA with IC_{50} value about 7 μM.

Compounds **67-69** (Table 4) exhibited relatively low furin inhibiting effect. Though, notably, they were able to inhibit the PCs activity, furin in particular, as it was found for iridoids **67**, **68** and quinone **69**.

6. Aromatic compounds containing positively charged substituents

It has previously been shown that the presence of one or more of guanidine groups in the structure of non-peptide inhibitors facilitated their interaction with the furin active site [38]. Given the structure of the enzyme binding site determined by X-ray analysis [49, 50], it could be expected that most effective inhibitors of furin would be extended low-molecular compounds. Moreover, these compounds should contain several positively charged substituents, which would provide effective interaction with Glu and Asp residues of the enzyme binding site.

Analysis of furin inhibition effect of aromatic compounds containing amidinohydrazone groups (Table 5, compounds **70-91**) [51-53] revealed that compounds with one positively charged group in molecule inhibited only slightly furin activity. The presence of two such groups resulted in more effective binding with the enzyme. It was assumed that one positively charged group might bind to the S_1 pocket of the enzyme, and the second one - to the S_2 sub-site. Substitution of hydrogen in amino group of compound **70** by positively charged groups (compounds **74-78**) led to increase in the inhibitory activity. The most efficient furin inhibitor, among the studied derivatives, contained two amidino-

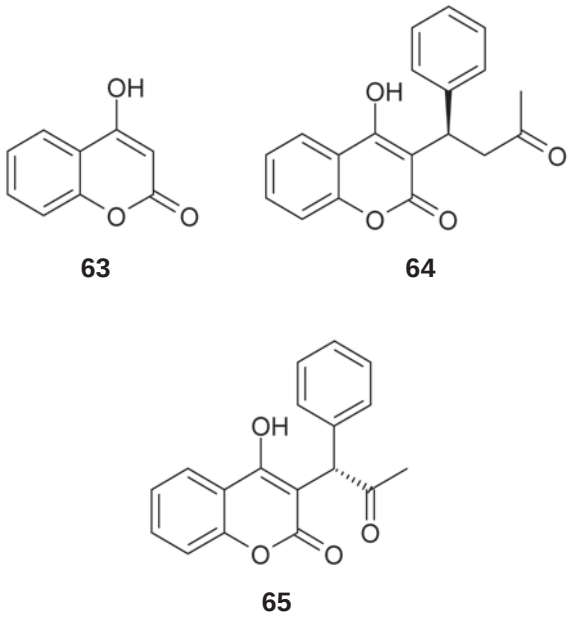


Fig. 8. Coumarin-based PCs inhibitors

hydrazone and one guanidine groups at the linker (compound **76**). Although derivative **80** with four amidinohydrazone substituents exhibited significant inhibition (K_i = 0.58 μM), it should be noted that increase in the number of positively charged groups to 4 did not significantly impact enzyme inactivation.

The most potent furin inhibitors among bis-amidinohydrazones **85-89**, which structures differ in linker nature, were found to be compounds **87**, **88** (K_i = 0.51-0.74 μM) with a hydrophobic benzene ring in the linker structure [52]. These findings were confirmed by data on the inhibitory properties of bis- guanidinophenyl esters (Fig. 3, compounds **28-33**) [40].

It was shown in work [54] that naphthofluorescein (known as CCG 8294) inhibited furin activity with a value of IC_{50} = 22 μM. The results of computer modelling and docking showed that its conformation in the enzyme active site was similar to conformation of baicalein (compound **46**) – well known furin inhibitor. Naphthofluorescein also blocked the matrix metalloprotease proMT1-MMP processing and aggressiveness of human fibrosarcoma cells (HT1080).

It was shown in 2015 that non-peptide furin inhibitors can be designed on the calix[4]arene platform [55]. This macrocycle CX3im derivative (Table 6), which contains positively charged N-methylimidazole groups at the upper rim, inhibited furin with K_i = 58μM.

Moreover, the magnitude of the effect depended on hydrophobicity of the groups localized at the lower rim of calixarene cup.

Thus, in recent years the different classes of compounds that are capable of inhibiting the PCs (furin in particular) activities have been established. It should be noted that the sources of PCs inhibitors have often been natural substances. Such substances have a wide spectrum of biological activity associated with their ability to inactivate enzymes.

It has been shown that potential furin inhibitors should contain several positively charged groups for effective interaction with negatively charged glutamic and aspartic amino acid residues on the surface of the enzyme binding pocket.

In general, effectiveness of furin inhibition by non-peptide compounds is inferior to power of bioengineered α1-PDX protein with K_i ~ 600 pM [56].

Table 4. Anti-furin activity of several natural compounds in the presence of a fluorogenic substrate Boc-RVRR-MCA

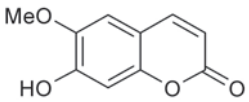
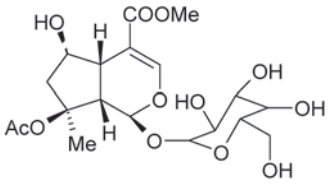
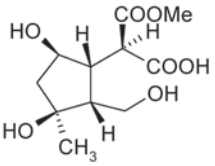
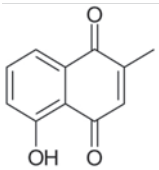
Compound	Structure	IC_{50}
66 Scopoletin		5 μM (at concentration of substrate 60 μM) 8 μM (at concentration of substrate 40 μM) 10 μM (at concentration of substrate 20 μM)
67 Barlerin		>100 μM
68 Shanzhiol		>80 μM
69 Plumbagin		>100 μM

Table 5. Furin inhibitors containing amidinohydrazone substituents at aromatic ring [51-53]

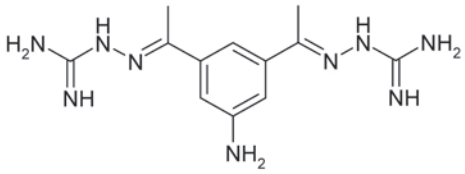
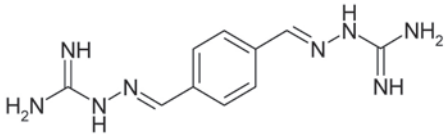
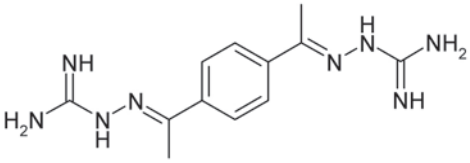
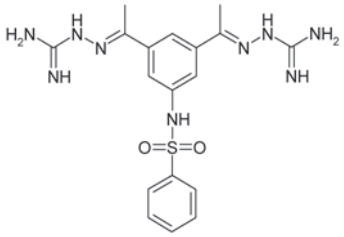
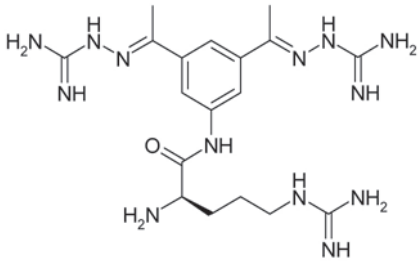
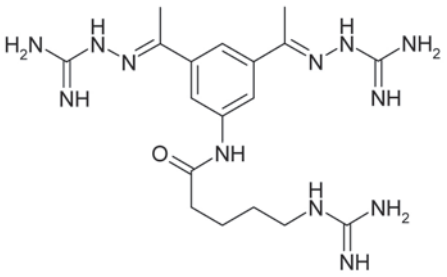
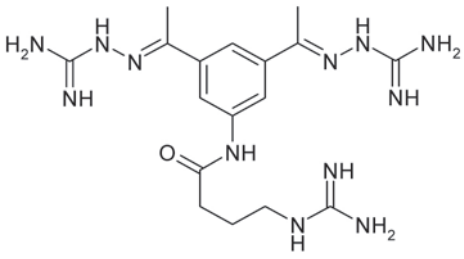
Compound	Structure	K_i, μM
70		1.82
71		1.47
72		1.84
73		1.55
74		1.13
75		1.04
76		0.46

Table 5. Continuation

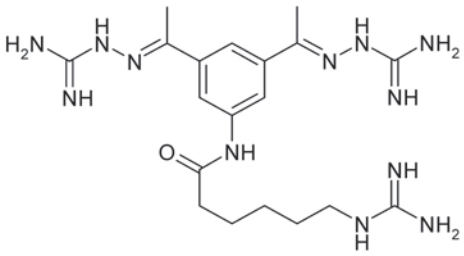
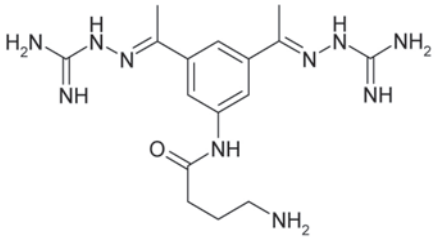
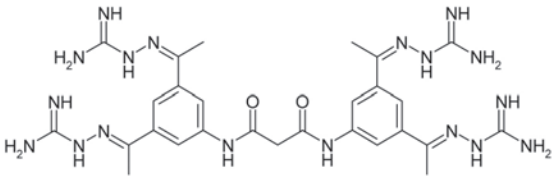
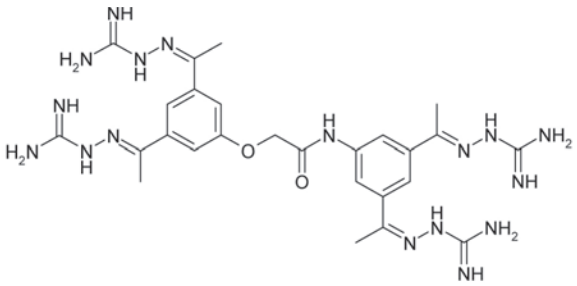
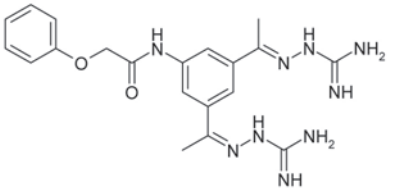
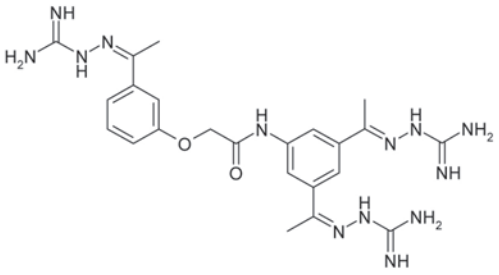
77		1.42
78		1.20
79		1.13
80		0.58
81		5.61
82		1.46

Table 5. Continuation

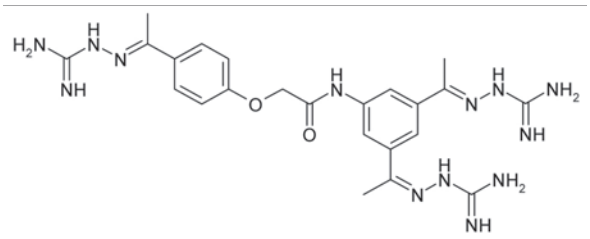
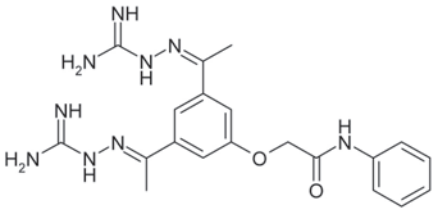
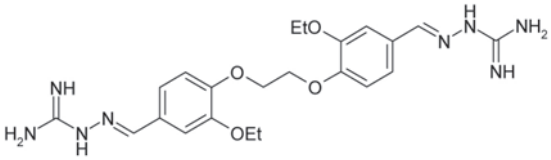
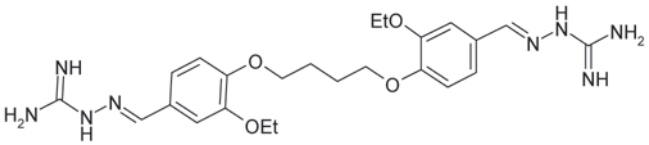
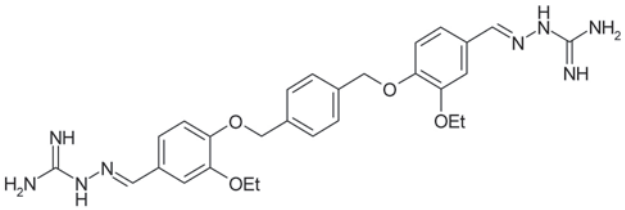
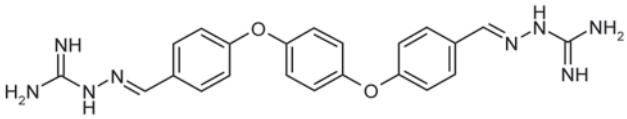
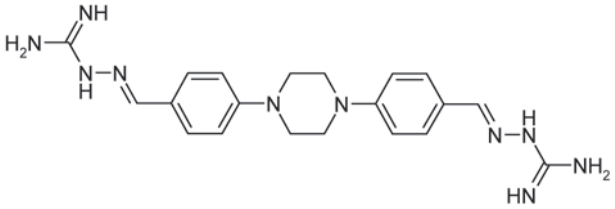
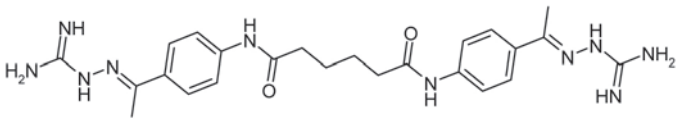
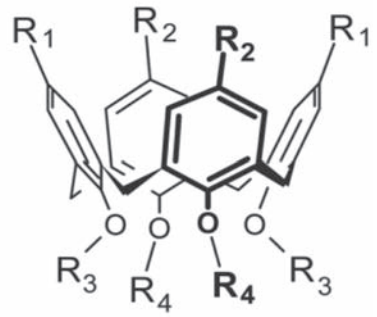
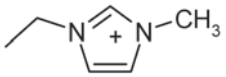
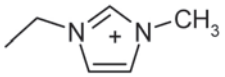
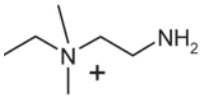
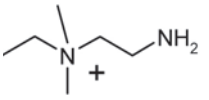
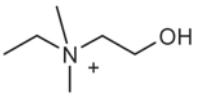
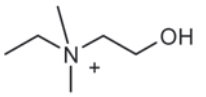
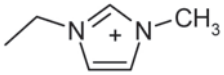
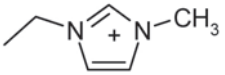
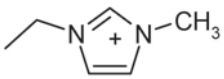
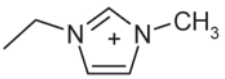
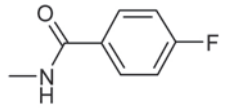
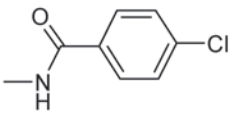
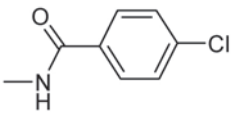
83		1.94
84		1.35
85		3.30 ± 0.10
86		1.10 ± 0.10
87		0.51 ± 0.02
88		0.74 ± 0.08
89		1.54 ± 0.48
90		1.21
91		3.90

Table 6. The structure of calix[4]arenes and their effect on furin activity at pH 7.3 [55]



Code	R_1–R_4 Structure				Inhibition of furin, %
	R_1	R_2	R_3	R_4	
CX3im			$-(CH_2)_2-CH_3$	$-(CH_2)_2-CH_3$	66.2±1.3
CX3N			$-(CH_2)_2-CH_3$	$-(CH_2)_2-CH_3$	59.8±1.0
CX3			$-(CH_2)_2-CH_3$	$-(CH_2)_2-CH_3$	45.8±2.4
CX8im			$-(CH_2)_7-CH_3$	$-(CH_2)_7-CH_3$	41.7±4.3
CX6im			$-(CH_2)_5-CH_3$	$-(CH_2)_5-CH_3$	24.2±3.9
C-100	$-C(CH_3)_3$		$-(CH_2)_2-CH_3$	H	22.1±0.3
C-89			$-(CH_2)_2-CH_3$	$-(CH_2)_2-CH_3$	18.4±4.3

In recent years, based on Phac-RVR-4-Amba, peptidomimetics **1** and **2**, the most effective synthetic competitive inhibitors of furin, have been developed (K_i ~ 5-8 pM) [35, 36]. Despite numerous publications concerning development and the study of the PCs inhibitors' properties, still only limited data on their clinical trials have been represented [57]. An important issue is the selectivity of these compounds, in particular, synthesis of compartment-specific furin inhibitors, and their safety to the human body. It is vital that these compounds would have greater stability, bioavailability and improved pharmacokinetic characteristics. Along these lines, the search for effective PCs inhibitors among low-molecular mass compounds remains a current area of research.

ХІМІЧНА БУДОВА ТА ВЛАСТИВОСТІ НИЗЬКОМОЛЕКУЛЯРНИХ ІНГІБІТОРІВ ФУРИНУ

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Огляд присвячено аналізу взаємозв'язку між хімічною будовою та властивостями низькомолекулярних сполук як інгібіторів фурину – найвивченішої пропротеїнконвертази, яка бере участь у розвитку таких патологічних станів, як онкологічні захворювання, вірусні та бактеріальні інфекції та ін. Розглянуто дані новітніх робіт про вплив пептидів і псевдопептидів, сполук з ароматичними або гетероциклічними кільцями, низки природних сполук: флавоноїдів, кумаринів та ін. на інактивацію ензиму. Показано, що ефективність блокування активності фурину зростає зі збільшенням кількості позитивно заряджених груп у структурі цих сполук. Встановлено, що найпотужнішими інгібіторами на сьогодні є пептидоміметики (K_i = 5–8 пМ). Проте жодна із синтезованих сполук не знайшла практичного застосування. Важливою лишається проблема селективності інгібіторів, покращення їхньої стабільності, біодоступності та безпечності для організму людини.

Ключові слова: фурин, синтетичні інгібітори, пептиди, псевдопептиди, похідні 2,5-дидезоксистрептаміну, енедіїни, амідиногідразони, флавоноїди, кумарини.

ХИМИЧЕСКОЕ СТРОЕНИЕ И СВОЙСТВА НИЗКОМОЛЕКУЛЯРНЫХ ИНГИБИТОРОВ ФУРИНА

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Обзор посвящен анализу взаимосвязи между химическим строением и свойствами низкомолекулярных соединений в качестве ингибиторов фурина – наиболее изученной пропротеинконвертазы, принимающей участие в развитии таких патологических состояний, как онкологические заболевания, вирусные и бактериальные инфекции и др. Рассмотрены данные новейших работ о влиянии пептидов и псевдопептидов, соединений с ароматическими или гетероциклическими кольцами, а также ряда природных соединений: флавоноидов, кумаринов и др. на инактивацию энзима. Показано, что эффективность блокирования активности фурина возрастает с увеличением количества положительно заряженных групп в структуре этих соединений. Установлено, что наиболее эффективными ингибиторами на сегодняшний день являются пептидомиметики (K_i = 5–8 пМ). Однако ни одно из синтезированных соединений не нашло практического применения. Важной остается проблема селективности ингибиторов, улучшение их стабильности, биодоступности и безопасности для организма человека.

Ключевые слова: фурин, синтетические ингибиторы, пептиды, псевдопептиды, производные 2,5-дидезоксистрептамина, энедиины, амидиногидразоны, флавоноиды, кумарины.

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