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Brief Article

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Novel Small Molecule Inhibitors of Activated Thrombin Activatable Fibrinolysis Inhibitor (TAFIa) from Natural Product Anabaenopeptin

Nis Halland,*[,†] Mark Brönstrup,[‡] Jörg Czech,[†] Werngard Czechtizky,[†] Andreas Evers,[†] Markus Follmann,[§] Markus Kohlmann,[†] Matthias Schiell,[†] Michael Kurz,[†] Herman A. Schreuder,[†] and Christopher Kallus*[,†]

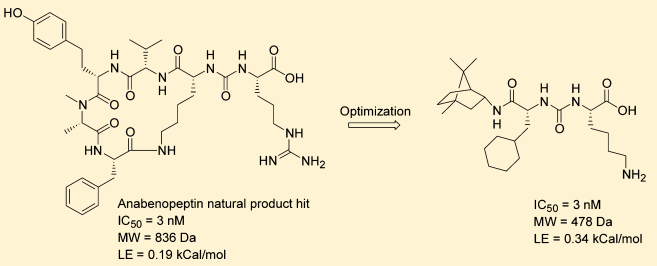
[†]Sanofi R&D, Industriepark Höchst Building G838, D-65926 Frankfurt am Main, Germany

[‡]Helmholtz Institute for Infection Research, Inhoffenstraße 7, D-38124 Braunschweig, Germany

[§]Bayer Healthcare, Aprather Weg 18A, D-42113 Wuppertal, Germany

Supporting Information

ABSTRACT: Anabaenopeptins isolated from cyanobacteria were identified as inhibitors of carboxypeptidase TAFIa. Cocrystal structures of these macrocyclic natural product inhibitors in a modified porcine carboxypeptidase B revealed their binding mode and provided the basis for the rational design of small molecule inhibitors with a previously unknown central urea motif. Optimization based on these design concepts allowed for a rapid evaluation of the SAR and delivered potent small molecule inhibitors of TAFIa with a promising overall profile.



INTRODUCTION

Drug discovery efforts in the area of thrombosis have historically focused on preventing coagulation by targeting proteases such as thrombin, factor Xa, or factor VIIa in the coagulation cascade or by addressing antiplatelet targets like P2Y12 or GPVI.[1] In contrast, approaches enhancing fibrinolysis by targeting other downstream enzymes such as factor XIIIa or TAFIa have received much less interest despite their potential advantages. As TAFIa inhibition neither interferes directly with coagulation nor acts on platelet function or influences other thrombin actions, it is expected to present a novel antithrombotic mechanism with a low associated risk of bleeding as opposed to established therapies such as vitamin K antagonists (warfarin, phenprocumon) or factor Xa inhibitors.

TAFI, a 423 amino acid protein synthesized in the liver and present in plasma as a zymogen, is activated by thrombin and converted to TAFIa by proteolysis at Arg92. The activated form cleaves arginine and lysine residues on, e.g., the surface of fibrin after initial degradation by plasmin. Therefore, TAFIa plays an important role in the balanced and coupled coagulation−fibrinolysis system. The arginine and lysine residues serve as binding sites for plasminogen and tissue plasminogen activator (tPA), and cleaving them off results in decreased plasmin formation, which protects fibrin clots from degradation. Inhibition of TAFIa increases plasmin formation and endogenous fibrinolysis, leading to an antithrombotic effect as described in several reviews.[2−5] TAFIa, a metallo carboxypeptidase, shows a high selectivity for the C-terminal basic amino acid side chains of proteins and peptides,[6−8] and the cleavage of the C-terminal peptide bond is catalyzed by coordination to a zinc ion present in TAFIa's active site.[9−11] Three features are essential for a potent active site inhibitor of TAFIa. First, a carboxylic acid group, or isostere, mimicking the C-terminal group, second, a basic group to achieve binding affinity and selectivity against other carboxypeptidases, and third, a zinc binding residue. Typical known TAFIa inhibitors contain zinc binders such as phosphinic acids,[12−14] carboxylic acids,[15] hydroxamic acids,[16,17] thiols,[18,19] and more recently imidazoles.[20] The majority of the previously reported TAFIa inhibitors are known to exhibit a high structural similarity and a steep SAR. Recently nanobody and monoclonal antibody TAFI inhibitors have also been reported.[21,22] Here we report the discovery of anabaenopeptins, macrocyclic secondary metabolites from cyanobacteria, as a novel class of TAFIa inhibitors, and their structure-guided rescaffolding to a novel lead series. While a part of the structures and activity data have been reported in a patent application,[23] the essential steps of lead generation, including initial discovery, successful reduction of structural complexity of the natural products, and the use of X-ray crystallography to explore the central urea motif, are disclosed.

RESULTS AND DISCUSSION

To enrich the Sanofi discovery compound collection with original, bioactive chemical matter, we have exploited cyanobacteria as a source of natural product-based leads.

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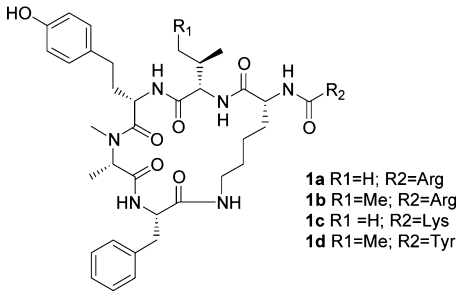
Because of their elaborate biosynthetic machineries,[24] cyanobacteria have proven to be producers of structurally diverse compounds that exhibit potent bioactivity against numerous target classes.[25,26] The isolation of pure compounds from an extract of *Planktothrix rubescens* through preparative chromatography yielded a series of macrocyclic peptides, inter alia anabaenopeptin B **1a**, anabaenopeptin F **1b**, anabaenopeptin C **1c**, and the structurally closely related oscillamide Y **1d**. Anabaenopeptins are featured by a cyclic pentapeptide core and an exocyclic C-terminal residue that is linked to the core via an ureido group (Table 1). A conserved D-lysine residue constitutes a central structural element that attaches the ureido group through its α-amino function and closes the cyclic pentapeptide through an isopeptide bond.

Table 1. IC_{50} Values of Anabaenopeptins on a Panel of Proteases/Carboxypeptidases (μM)



	TAFIa	CPA	CPN	FXa	FVIIa	FIIa	FXIa
1a	0.0015	3.9	5.4	>100	>100	1.6	24
1b	0.0015	1.1	2.7	38	35	0.35	3.9
1c	0.0019	>100	5.7	>100	>100	6.0	12.0
1d	0.40	nd	nd	nd	nd	nd	nd

Because it is known from literature that similar anabaenopeptins exhibit carboxypeptidase-inhibiting properties[27,28] and marine natural products with a similar peptide core have been reported previously,[29] we decided to test our analogues for their inhibitory effect on TAFIa. To our delight, several members of this natural product family were found to be potent inhibitors of TAFIa. Moreover, other targets in the coagulation cascade were not influenced significantly (Table 1). For example, **1a** exhibited an IC_{50} of 1.5 nM against TAFIa with a >500-fold selectivity over carboxypeptidases A and N (CPA, CPN), FXa, FVIIa, FIIa, and FXIa. An equal activity profile was obtained for **1b**, which differs from **1a** by a Val to Ile replacement. Anabaenopeptin C **1c**, an analogue that can be formally derived from **1a** by replacing the C-terminal arginine residue with lysine, displayed a similar potency (IC_{50} = 1.9 nM) against TAFIa. In contrast, a pronounced decrease in activity was observed for oscillamide Y **1d** (IC_{50} = 400 nM), an analogue with a C-terminal tyrosine residue. Although TAFIa inhibition and eADME data recorded for anabaenopeptins were very promising, a lead optimization of the natural product was not attempted for several reasons. First, the access to bulk material by either fermentation or total synthesis was considered to be elaborate, and second, the oral bioavailability of anabaenopeptins was not surprisingly found to be low. Furthermore, the optimization of complex natural product structures is challenging and often unsuccessful. We initiated our search for simplified structural analogues by attempting to obtain cocrystal structures of anabaenopeptins in the surrogate protease carboxypeptidase B (CPB) in order to understand the essential interactions between anabaenopeptins and TAFIa.

Porcine CPB was used instead of TAFIa, as the latter is highly unstable with a half-life of about 2 h at 22 °C.[30] CPB, on the other hand, is stable and homologous to TAFIa, with 48% sequence identity and 0.8 Å rms difference in Cα positions.[31,32] Furthermore, it has been found that the binding affinity of several inhibitors is very similar for both enzymes, and for **1a** the affinity to CPB is IC_{50} = 0.2 nM. A schematic representation and picture of the detailed interactions between anabaenopeptin B **1a** and CPB are shown in Figure 1. From

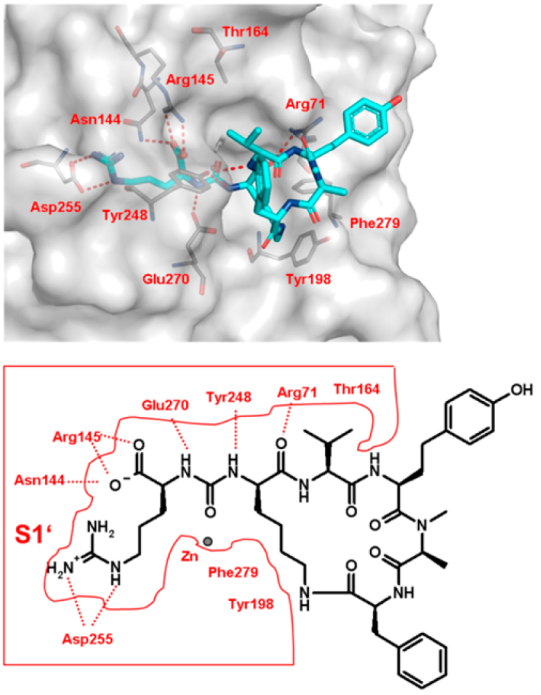


Figure 1. Crystal structure of **1a** in complex with CPB.

this X-ray cocrystal structure, it is clear that the polar groups on **1a**, C-terminal arginine, carboxylate group, and urea- and amides are closely matched by compatible polar H-bonding groups in the enzyme active site, e.g., Asp255 in the S1′ pocket, Asn144 and Arg145 in the carboxylate binding site, and Glu270 and Tyr248 in the active site entrance, as well as at the catalytic zinc. The aliphatic part of the D-lysine was also found to make van der Waals contacts with the side chains of Tyr198 and Phe279, while the valine residue contacts the methyl group of Thr164. The remaining parts of the **1a** did not show any interactions with the protein. It is clear from the CPB−**1a** cocrystal that a large part of **1a** remained outside the binding pocket and did not interact with CPB. We therefore speculated that omitting this part of the molecule should not lead to a significant loss of activity and provide access to an entirely novel class of small molecule inhibitors. To test this hypothesis, we synthesized the simple noncyclic derivatives **2** and **3a** (Scheme 1, Table 2).

Despite a reduction in molecular weight by almost a factor of 2 and the fact that opening of the macrocyclic system introduced many degrees of freedom, both analogues still exhibited a single-digit micromolar inhibitory activity toward TAFIa. The ligand efficiency (LE) was also significantly improved by deleting nonbinding and solvent exposed residues (Scheme 1).

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Scheme 1. First Small Molecule TAFIa Inhibitors Derived from Anabaenopeptin B 1a

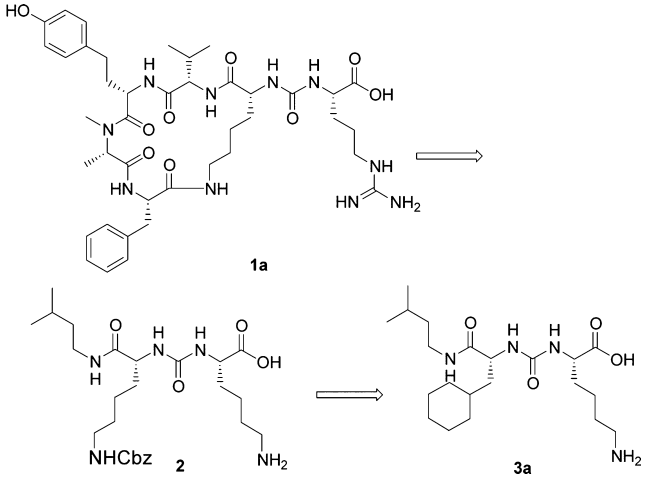


Table 2

	IC_{50} (μM)	MW	LE (kcal/mol)
1a	0.0015	836	0.19
2	1.4	521	0.22
3a	1.5	412	0.27

These results prompted us to further investigate the potential of urea based small molecule inhibitors by library synthesis. A cocrystal structure of **3a** in CPB (Figure 2, PDB 4uia)) was

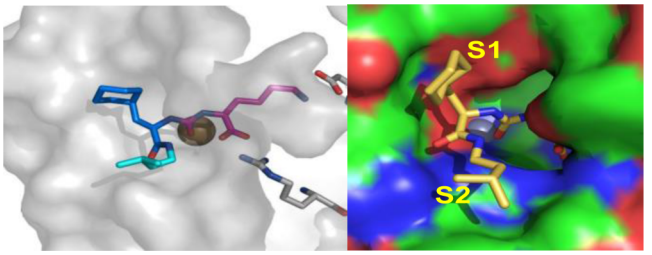
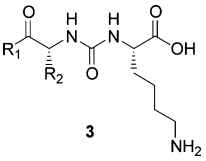
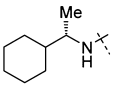
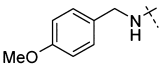
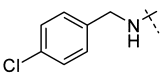
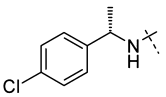
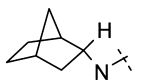
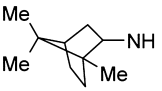


Figure 2. Crystal structure of **3a** in complex with CPB. Both views are down the entrance to the active site. The specificity pockets S1 and S2 are indicated. The S1′ specificity pocket is located inside the protein (PDB ID 4uia).

used as the basis for the rational, computer-aided design of a library of amide derivatives to first explore the hydrophobic S2 pocket by variation of the amide residue R1 and the amino acid side chain R2 (Table 3). All ureas in Tables 3 and 4 were synthesized as depicted for compound **3c** in Scheme 2. The commercially available (*R*)-*N*-Boc-cyclohexylalanine was deprotected using trifluoroacetic acid in dichloromethane. A 1,1′-carbonyldiimidazole mediated urea coupling of (*R*)-cyclohexylalanine and commercially available H-Lys(Boc)-OtBu afforded **4**, which served as a precursor for library synthesis. Building block **4** was coupled with cyclohexylamine using 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDC·HCl), 1-hydroxybenzo-triazole, and *N*-methyl-morpholine in a 3:1 mixture of dichloromethane and DMF to afford the protected product **5**. Boc-deprotection and tBu-ester cleavage using trifluoroacetic acid in dichloromethane delivered product **3c** that was purified by preparative RP-HPLC. To evaluate the SAR around R1, a library of lysine derived ureas was prepared

Table 3. TAFIa Inhibitory Activity of Lysine Derived Ureas 3



	R1	R2	IC_{50} (μM)
3a	*i*-Pent-NH-	*c*-Hex-CH_2	1.5[c]
3b		*c*-Hex-CH_2	59
3c	*c*-Hex-NH-	*c*-Hex-CH_2	0.17
3d	3-methylpentan-2-NH	*c*-Hex-CH_2	0.19
3e	*i*-Bu-NH-	*c*-Hex-CH_2	0.85
3f[a]	*t*-Bu-CH_2NH-	*c*-Hex-CH_2	0.69
3g	(*R*)-3-methylbutan-2-NH	*c*-Hex-CH_2	0.39
3h		*c*-Hex-CH_2	0.13
3i	1-adamantan-NH	*c*-Hex-CH_2	0.031
3j	2-adamantan-NH	*c*-Hex-CH_2	0.019
3k	Ph_2CHCH_2NH	*c*-Hex-CH_2	0.12
3l		*c*-Hex-CH_2	0.90
3m		*c*-Hex-CH_2	1.2
3n		*c*-Hex-CH_2	0.11
3o		*c*-Hex-CH_2	0.039
3p	 (*R*)-(+)-bornyl	*c*-Hex-CH_2	0.003[d]
3q	(*R*)-(+)-bornyl	Bn	0.015
3r	(*R*)-(+)-bornyl	*i*-Bu	0.057
3s	(*R*)-(+)-bornyl	*i*-Pr	0.46
3t	(*R*)-(+)-bornyl	*c*-Hex	1.0
3u	(*R*)-(+)-bornyl	H	3.7
3v	(*R*)-(+)-bornyl	$(CH_3)_2$	6.1
3w	(*R*)-(+)-bornyl	propyl	0.015
3x	(*R*)-(+)-bornyl	*c*-propyl-CH_2	0.007
3y	(*R*)-(+)-bornyl	*c*-butyl-CH_2	0.009
3z[b]	(*R*)-(+)-bornyl	*c*-pentyl-CH_2	0.004

[a]Mixture of diastereomers. [b]Racemic at cyclopentylmethylene chiral center. [c]CPB IC_{50} = 0.19 μM. [d]CPB IC_{50} = 0.001 μM

using various amines and selected examples are shown in Table 3. In general, only moderate activity was obtained with amides derived from secondary amines, e.g., compound **3b**, whereas primary alkylamines or α-branched amines delivered more potent compounds **3a**, **3c**–**n**. The most potent inhibitors were found when the steric bulk of R1 was increased by using large bi- or tricyclic cycloalkyl residues such as adamantane **3i,j**, norbornyl **3o**, or bornyl **3p**. The (*R*)-(+)-bornylamide **3p** proved to be the most potent compound in this series and therefore served as a lead for further investigation. Crystal

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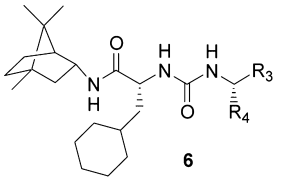
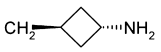
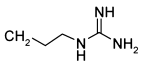
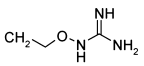
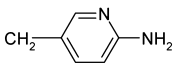
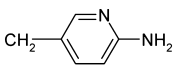
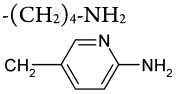
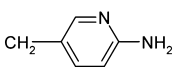
Table 4. TAFIa Inhibitory Activity of Ureas 6

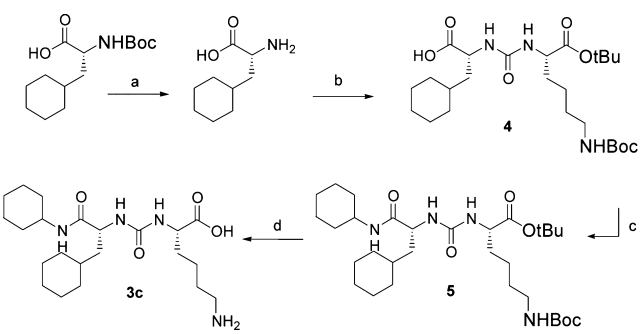


6

	R3	R4	IC_{50} (μM)
6a	$-CO_2H$	$-(CH_2)_3-NH_2$	0.018
6b[a]	$-CO_2H$		0.11
6c	$-CO_2H$	$-CH_2SO_2(CH_2)_2NH_2$	0.038
6d	$-CO_2H$	$-CH_2S(CH_2)_2NH_2$	0.004
6e	$-CO_2H$		0.004
6f	$-CO_2H$		0.10
6g	$-CO_2H$		0.001
6h[b]	$-CO_2H$		0.25
6i	$-CONH_2$	$-(CH_2)_4-NH_2$	0.75
6j	$-CONH_2$		0.57
6k	-CN		54

[a]Norbornyl amide instead bornylamide. Racemic *trans*-2-amino-3-(3-aminocyclobutyl)propanoic acid employed. [b](*S*)-Configuration at the carboxylic acid chiral center.

Scheme 2. Synthesis of Ureas 3[a]



[a]Reagents and conditions: (a) TFA, CH_2Cl_2, 20 °C; (b) H-Lys(Boc)-OtBu, 1,1′-carbonyldiimidazole, triethylamine, DMF, 80 °C; (c) cyclohexylamine, *N*-methyl-morpholine, 1-hydroxy-benzotriazole, 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride, CH_2Cl_2/DMF, 20 °C; (d) TFA, CH_2Cl_2, 20 °C.

structure determination suggests that the high affinity of **3p** is due to a high shape complementarity of the (*R*)-(+)-bornyl side chain with the hydrophobic S2 pocket, as shown in Figure 3a. For this cocrystal structure (PDB ID 4uib), we used "TAFInized" CPB (tafCPB), in which eight residues in the active site of CPB were replaced with the corresponding residues from TAFI (see Supporting Information section 2). As shown in Figure 3b, this crystal structure confirmed the binding mode of **3p** to be very similar to the binding mode of anabaenopeptin B **1a**.

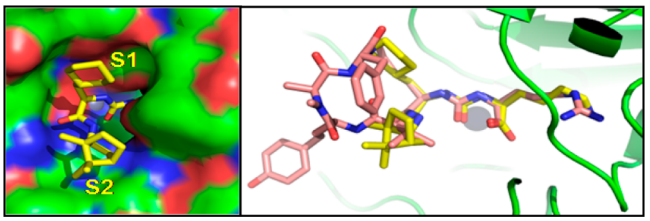


Figure 3. (a) Crystal structure of the **3p**−tafCPB complex (PDB ID 4uib). (b) Superposition of the tafCPB−**3p** (yellow) and the CPB−**1a** (pink) complex.

We then turned our attention to the SAR of the amino acid side chain R2. The size of R2 plays a crucial role in obtaining high affinities. Small substituents such as, e.g., H (**3u**), dimethyl (**3v**), or α-branched substituents (**3s**,**t**), afforded only moderate TAFIa inhibitors. On the other hand, β-branched residues such as methylene cycloalkyl (**3p**, **3x**−**z**) or medium-sized residues such as benzyl (**3q**), *iso*-butyl (**3r**), or propyl (**3w**) afforded highly potent TAFIa inhibitors with activity in the low nanomolar range. In a next step, we explored the SAR, and attempted to replace the acidic R3 and the basic R4 moieties (Table 4). The lysine side chain was replaced with several other basic aminoalkyl residues such as the ornithine side chain (**6a**) or with the *trans*-aminocyclobutyl residue (**6b**). This resulted in a slightly decreased activity, whereas replacement with arginine (**6e**) did not change the activity. Introduction of a sulfur atom in the lysine side chain (**6d**) did not alter the activity, but oxidation to the sulfone had a negative impact (**6c**). Replacement of the lysine side chain by an aminopyridyl methylene residue, a less basic lysine side chain bioisostere,[9] afforded the so far most active TAFI inhibitor **6g** with an IC_{50} of 1 nM. The opposite enantiomer having the (*S*) configuration at the carboxylic acid chiral center (**6h**) was, as expected, significantly less active, as the spatial configuration is opposite to the one found in naturally occurring amino acids. We also attempted to replace the carboxylic acid with a cyano or primary amide moiety, but both resulted in a significant loss of activity **6i**−**j**.

The hydrochloride salt of the potent urea **3p** was selected for further in vitro eADMET and physicochemical profiling to assess its potential as preclinical candidate (Table 5). **3p** is a strong TAFI inhibitor with an IC_{50} of 3 nM, and only a minor decrease in activity was observed in the presence of 1% human serum albumin.The high activity of **3p** was reflected in a ligand efficacy (LE) of 0.34 kcal/mol and very high lipophilic ligand efficacy (LLE) of 6.3. **3p** is also relatively polar with a Log *D* = 2.2 and possesses a very high aqueous solubility (Table 5). Furthermore, it was found to be reasonably stable when incubated with human, mouse, or rat microsomes, showed a very moderate intrinsic clearance in human hepatocytes, and an intermediate CYP3A4 inhibition with an IC_{50} = 6.5 μM at the midazolam binding site. **3p** did not display any CYP1A1, CYP1A2, or CYP3A4 induction, and no hERG channel liability was found. Lastly, **3p** was tested against a panel of other proteases at 100 μM concentration, and none of them were inhibited to a significant degree. Because of the low Caco-2 permeability and high solubility, an intravenous application was considered the preferred route of administration for in vivo studies.

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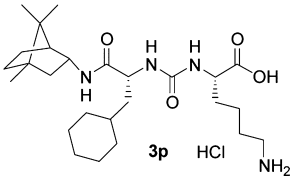
Table 5. Physicochemical and in Vitro ADMET Properties of Urea 3p



property	3p
MW (parent)	478.67
solubility mg/mL (pH = 7.4, 25 °C)	0.443
Log *D* (pH 7.4, 25 °C)	2.22
ClogP	4.16
PSA (Å^2)	134
H-bond donors	6
H-bond acceptors	8
rotatable bonds	12
LE(kcal/mol)/LLE	0.34/6.3
IC_{50} TAFIa (μM)	0.003
IC_{50} TAFIa (μM) + 1% human serum albumin	0.007
metabolic degradation in human microsomes (%)[c]	31
metabolic degradation in rat microsomes (%)[c]	4
metabolic degradation in mouse microsomes (%)[c]	1
Caco-2 permeability ($\times 10^{-7}$cm/s)	1.8 (low)
CYP3A4 Inhibition IC_{50} (μM) midazolam site/testosterone site[a]	6.5/15
hERG channel inhibition IC_{50}, patch clamp (μM)[b]	>30
other peptidases % inhibition at 100 μM concentration FIIa, FVIIa, FXa, FXIa, FXIIa, urokinase, C 1s, tPA, tryptase, trypsin, plasmin, KLKB1[d]	<30%

[a]Incubated at 37 °C for 10−30 min at 0.3−30 μM.[27] [b]Patch-clamp technique in the whole-cell configuration on recombinant chinese hamster ovary (CHO) cells. [c]% degradation after 20 min incubation (see Supporting Information for details). [d]Kallikrein B, plasma (Fletcher factor) 1.

CONCLUSION

In summary, we have developed a new type of highly active urea small molecule inhibitors of TAFIa by exploiting a novel zinc-binding motif. Starting from the X-ray cocrystal structure of macrocyclic natural product anabaenopeptin, drug design efforts lead to simplified structures with reduced molecular weight and increased ligand efficiency. Further X-ray cocrystal structures of small molecule inhibitors in CPB were obtained to guide the lead optimization program which resulted in the highly active urea **3p**. Compound **3p** displayed a promising in vitro profile and is a suitable candidate for further in vivo studies.

EXPERIMENTAL SECTION

Chemistry. ^{1}H NMR spectra were recorded in the indicated deuterated solvent at 400 or 500 MHz. Purity of all compounds tested in biological assays were determined to be >95% by LCMS.

All ureas were prepared following a three-step procedure as described for (2*S*)-6-amino-2-[[(1*R*)-2-(cyclohexylamino)-1-(cyclohexylmethyl)-2-oxo-ethyl]carbamoylamino]hexanoic acid trifluoroacetate **3c**. To a solution of commercially available (*R*)-2-*tert*-butoxycarbonylamino-3-cyclohexyl-propanoic acid (3.0 g, 11.1 mmol) in 20 mL of dichloromethane was added 5 mL of trifluoroacetic acid, and the mixture stirred at 20 °C for 14 h. The mixture was evaporated, and 50 mL of H_2O was added to the remaining solid and the mixture was lyophilized to give 2.84 g (90%) of (*R*)-2-amino-3-cyclohexyl-propanoic acid trifluoroacetate as a colorless solid that was used in the next step without further purification.

(2*S*)-6-Amino-2-[[(1*R*)-2-(cyclohexylamino)-1-(cyclohexylmethyl)-2-oxo-ethyl]carbamoylamino]hexanoic Acid 3c. A solution of ((2*R*)-2-[[(1*S*)-1-*tert*-butoxycarbonyl-5-(*tert*-butoxycarbonylamino)pentyl]carbamoylamino]-3-cyclohexyl-propanoic acid **4**, 80 mg, 0.16 mmol) and cyclohexylamine (23 mg, 0.16 mmol) in 3 mL of dichloromethane and 1 mL of DMF were treated with *N*-methyl-morpholine (53 μL, 0.48 mmol), 1-hydroxy-benzotriazole (28 mg, 0.21 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (37 mg, 0.19 mmol). The mixture was stirred at 20 °C for 14 h and extracted with dichloromethane/water. The organic phase was dried over $MgSO_4$ and evaporated. The crude product was dissolved in 4 mL of dichloromethane and 1.5 mL of trifluoroacetic acid was added, and the reaction stirred at 20 °C for 10 h. Preparative RP-HPLC using acetonitrile/water with 0.5% TFA as the eluent afforded 19 mg (22%) of **3c** as its trifluoroacetate salt. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 0.77−0.90 (m, 2H), 1.04−1.40 (m, 14H), 1.45−1.74 (m, 14H), 2.75 (sextet, 2H, J = 6.0 Hz), 4.07 (dt, 1H, J = 5.9, 8.2 Hz), 4.14 (q, 1H, J = 6.5 Hz), 6.20 (d, 1H, J = 8.8 Hz), 6.35 (d, 1H, J = 8.4 Hz) 7.64 (br, 3H), 7.78 (d, 1H, J = 8.0 Hz), 12.56 (br, 1H). MS (ES−) calcd: $[M - H]^-$, 423.30; found, 423.44.

(2*R*)-2-[[(1*S*)-1-*tert*-Butoxycarbonyl-5-(*tert*-butoxycarbonylamino)pentyl]carbamoylamino]-3-cyclohexyl-propanoic Acid 4. Commercially available (*S*)-2-amino-6-*tert*-butoxycarbonylamino-hexanoic acid *tert*-butyl ester hydrochloride (1.95g, 5.75 mmol) in 30 mL of DMF was treated with Et_3N (0.8 mL, 5.75 mmol) and 1,1′-carbonyl-diimidazole (0.93g, 5.75 mmol). The mixture was stirred at 20 °C for 30 min. (*R*)-2-Amino-3-cyclohexyl-propanoic acid trifluoroacetate, 1.64g, 5.75 mmol) and Et_3N (1.6 mL, 11.5 mmol) were added and the mixture heated to 80 °C to complete conversion of the intermediary imidazolide. Purification by flash chromatography on silica gel (using dichloromethane/methanol as the eluent) afforded 2.1 g (73%) of **4**. MS (ES +) calcd $[M + H]^+$, 500.33; found, 500.33.

ASSOCIATED CONTENT

Supporting Information

Representative experimental procedures for the isolation of anabaenopeptins, X-ray crystallography, synthesis, biochemical assays, and analytical data for all compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jm501840b.

AUTHOR INFORMATION

Corresponding Authors

*For N.H.: phone, +49-69305-36193; E-mail, Nis.Halland@sanofi.com.

*For C.K.: phone, +49-69305-20980; E-mail, Christopher.Kallus@sanofi.com.

Notes

The authors declare no competing financial interest.

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