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Chlorothiophenecarboxamides as P1 surrogates of inhibitors of blood coagulation factor Xa

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Abstract—Neutral chlorothiophenecarboxamides bearing an amino acid and a substituted aniline were synthesized and investigated for their factor Xa inhibitory activity in vitro. From selected 2-methylphenyl morpholinones the solution properties were determined. The most soluble and active compounds were then investigated in different animal species to compare the pharmacokinetic parameters. This led to a potent, water soluble and orally bioavailable candidate for further development: EMD 495235.

1. Introduction

The serine protease factor Xa (fXa) plays a crucial role in the coagulation cascade and has, therefore, been identified as an attractive target for the development of antithrombotic agents.[1] Inhibition of this central enzyme within this cascade has emerged as a particularly active area of research.

At the outset of the research obtaining inhibitors of fXa the reported compounds contained mono- or bis-(benz)amidine functionalities.[2] However, (benz)-amidine-containing molecules displayed undesired poor pharmacokinetic properties. In an effort to develop fXa inhibitors with more favourable oral profiles, we have discovered a series of potent nonamidines as exemplified by compound **1**[3] (Fig. 1), which contains a 4-chlorophenyl-carbamoyl P1 ligand, a central D-amino acid linker and a phenyl morpholinone P4 residue. The search for further novel benzamidine mimics led to the discovery of achiral chlorothiophene benzimidazoles such as compound **2**[4] (Fig. 1), which incorporated an alkylamide tether at the 5(6)-position of the benzimidazole ring together with a substituted phenyl morpholinone as P4 ligand. Although both compounds displayed fXa inhibitory activity in the mono-digit nanomolar range, their physicochemical parameters such as solubility (<10 μg/mL in pH 7 phosphate buffer) and oral pharmacokinetic profiles (bioavailabilty <10% in rats) have to be improved.

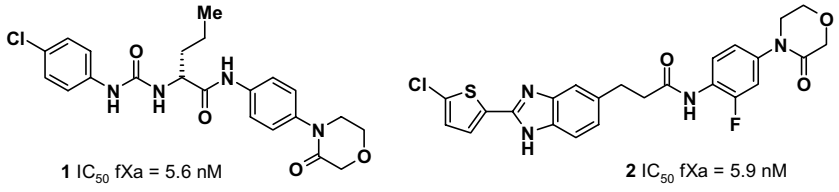

Figure 1. FXa binding affinities of chlorophenyl urea **1** and chlorothiophene benzimidazole **2**.

Keywords: Anticoagulants; Factor Xa inhibitors; Chlorothiophenecarboxamides; Pharmacokinetics; EMD 495235.
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To overcome this gap between the pharmacodynamics and pharmacokinetics an alternative route was envisaged starting from chlorothiophene.

A superimposition of representative fXa inhibitors of both classes of compounds based on the conformation found by co-crystallization of those inhibitors with fXa[4] showed, that the chlorothiophene residue is a good isoster for the substitution of the chlorophenyl ring in the S1 pocket (e.g., compound **1**). However, the urea moiety seemed to be not the optimal linking element between P1 residue and amino acid part. Computer modelling studies showed, that a methanone group could be a good replacement for the carbamoyl part within the chlorophenyl derivatives giving rise to chlorothiophenecarboxamides.

Recently, the 5-chlorothiophene–methanone fragment as a surrogate for the P1 moiety has also been described in the literature, although with different central scaffolds and P4 residues.[5]

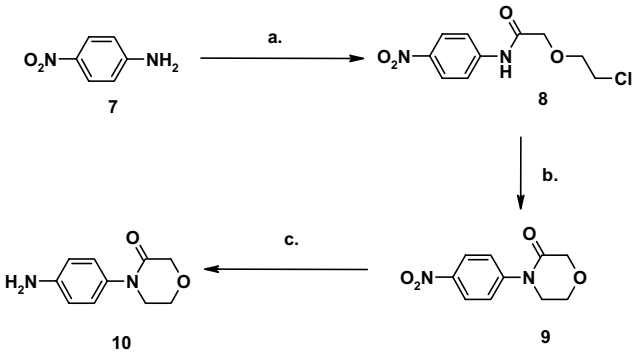
Herein, we report on structure–activity relationship studies around variations on the amino acid core as central scaffold and the P4 residue. Further studies of selected compounds on water solubility and pharmacokinetic parameters are presented resulting in the discovery of EMD 495235.

2. Chemistry

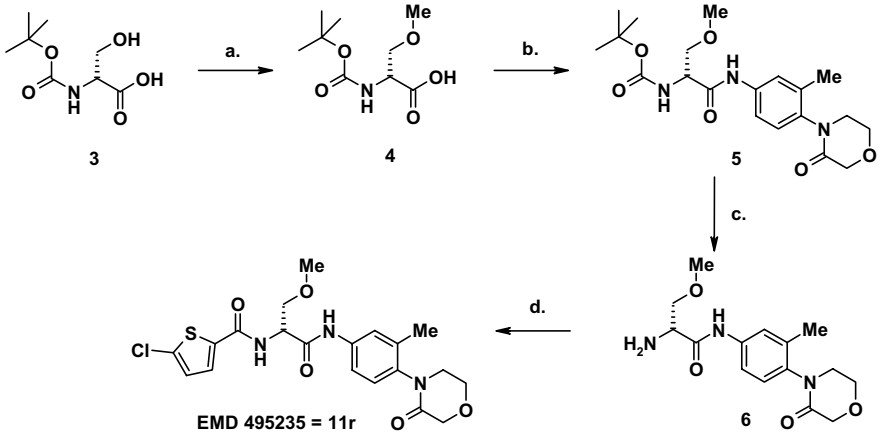
Starting with commercially available *N-tert*-butoxycarbonyl (Boc) protected amino acids the targeted compounds **11a**–**x** bearing a P1 chlorothiophenecarboxamide, an amino acid as core structure and the respective P4 anilino side chain were synthesized in three or four steps depending on the employed amino acid. A representative synthesis of the modified inhibitors **11a**–**x** is described in Scheme 1, exemplified by morpholinone **11r** (EMD 495235).

N-Boc-*O*-methyl-D-serine **4** was prepared in one step from the corresponding D-serine **3** by using methyl iodide and sodium methoxide in tetrahydrofuran.[6] Coupling of the resulting acid **4** and 4-(4-amino-2-methyl-phenyl)-morpholin-3-one under standard peptide-coupling techniques generated the amide **5**. The *N*-Boc protecting group was subsequently removed by treatment with trifluoroacetic acid in dichloromethane to give the amine **6**. Finally, intermediate **6** was converted with 5-chloro-thiophene-2-carboxylic acid again under peptide-coupling conditions to the targeted compound EMD 495235.

Scheme 2 illustrates a representative synthesis of prototypic P4 morpholinone building block **10**. Commercially available 4-nitro-phenylamine **7** was subjected to a standard acylation procedure with (2-chloro-ethoxy)-acetyl chloride[7] to give anilide **8**. Ring closure of this intermediate with potassium carbonate in acetonitrile afforded the corresponding morpholinone **9**. Finally, the nitro group of compound **9** was reduced under hydrogenation conditions to the desired anilino morpholinone **10**.



Scheme 2. General synthesis of the P4 morpholinones, illustrated at 4-(4-amino-phenyl)-morpholin-3-one **10**. Reagents and conditions: (a) (2-chloro-ethoxy)-acetyl chloride, toluene, 95 °C, 5 h; (b) K_2CO_3, MeCN, rt, 8 h; (c) H_2, Pd–C-5%, MeOH, 1.0 bar, 35 °C, 1 h.



Scheme 1. General synthesis of the final compounds **11a**–**x**, illustrated at EMD 495235 (=**11r**). Reagents and conditions: (a) NaOMe, MeI, THF, rt, 24 h; (b) 4-(4-amino-2-methyl-phenyl)-morpholin-3-one, EDC, HOBt, DMF, rt, 18 h; (c) TFA, DCM, rt, 2 h; (d) 5-chloro-thiophene-2-carboxylic acid, EDC, HOBt, DMF, rt, 24 h.

3. Results and discussion

The structure–activity relationships surrounding the inhibitors incorporating the different side chains of the amino acids and the modified P4 ligands are outlined in Table 1. Compounds **11a**–**x** were assayed against human fXa, thrombin and trypsin as previously described.[8] Briefly protease activity was monitored in vitro using protease-specific chromogenic substrates. Antiprotease activity of drugs was calculated from the OD ratio of drug and vehicle containing assay. For thrombin and trypsin all compounds displayed $IC_{50} > 10\,\mu M$.

2-Amino-acetamide **11a** containing the unsubstituted phenyl morpholinone **10** and a chlorothiophene–methanone was initially evaluated as an inhibitor of factor Xa. Although already submicromolar in binding affinity, this achiral compound delivered the weakest potency within our series. However, this result encouraged us to explore a variety of different substituents at the C-2 position of the central amino acid unit. Based on the findings within our chlorophenylurea series D-amino acids were employed at the beginning. A selection of this chosen derivatives is summarized in Table 1. Introduction of aliphatic C-2 side-chains markedly increased potency. The *n*-propyl substituent (**11d**) was the most effective, which displayed a 58-fold potency improvement compared to the parent **11a**. The corresponding C-2 methyl (**11b**) and ethyl (**11c**) side-chain derivatives were less active than the norvaline **11d**. Steric crowding closer to the C-2 branching point (**11e**) resulted in decreased fXa inhibitory activity. Replacement of the isopropyl group of **11e** with isobutyl **11f** regained potency, indicating that a larger group more apart from the branching point is still tolerated. Leucine **11f** is equipotent to the corresponding norvaline **11d**.

Replacement of the alkyl groups in **11a**–**f** with an aromatic phenyl ring resulted in the two phenylglycines **11g** and **11i**, respectively, depending on the employed chiral amino acid. Both *R*- and *S*-enantiomers were equipotent but showed a slight loss in binding compared to leucine **11f**. However, there is a preference for *R*-configuration at C-2 in aliphatic substituted inhibitors. The *S*-configurated isomer **11i** is about nine times less potent than *R*-leucine **11f**.

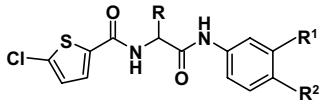
In order to support our assumption that a methanone is more favourable as a linker in 2-chlorothiophenes than a carbamoyl moiety, the urea analogue of D-norvaline **11d** was synthesized. Binding affinity dropped about 16-fold compared to that of the corresponding methanone **11d** (data not shown in Table 1), [9] which confirmed our initial hypothesis.

To explore P4 modification morpholinone in **11f** was replaced with different carbonyl bearing heterocycles. Two promising derivatives are shown in Table 1. With pyrazinone **11k** we observed a 2-fold loss in binding affinity, whereas pyridinone **11l** is equipotent to the parent **11f**. In general, pyridones could not compete with morpholinones with regard to pharmacokinetic properties within our different series. Therefore, the phenyl morpholinone became the focus of further efforts. To probe additional interactions in the S4-pocket the effects of substitution on the phenyl ring relative to the morpholinone were investigated. The results of *ortho*-substitution are shown in Table 1, which gave the best improvements concerning fXa binding. The initial compound **11m** in this series contained the isobutyl group in C-2 position of the central moiety and the *ortho*-methyl on the phenyl ring, which directly led to a 2-fold increase in potency compared to **11f**. The replacement of the isbobutyl in compound **11f** by a propyl (**11n**), an ethyl (**11o**) or a methyl (**11p**) resulted in slightly diminished binding affinities. However, introducing more polar C-2 side-chains based on oxygen further potency enhancement was observed. Compared to isobutyl **11f** the hydroxymethyl analogue **11q** displayed a 2-fold decreased affinity in binding, whereas the corresponding methylserine **11r** showed enhanced potency representing the most potent chlorothiophenecarboxamide inhibitor. Isosteric replacement of the methyl group in **11r** with a trifluoromethyl (**11s**), a chloro (**11t**) or a fluoro (**11u**) resulted in decreased binding affinities. Larger *ortho*-alkyl substituents than methyl were not prepared due to synthetic reasons. Further C-2 variations of the chain length based on the oxygen atom had a negative effect on fXa binding. Elongation of serine **11q** at the hydroxyl function with ethyl (**11v**), propyl (**11w**) or methoxyethyl (**11x**) decreased potency up to 2-fold.

Next, the physicochemical profile of the more potent 2-methylphenyl morpholinones was examined. The results of some selected inhibitors **11m**, **11o**–**r**, **11w** and **11x** are summarized in Table 2. The compounds are arranged in descending order of the octanol–water distribution coefficient ($\log D$ at pH 7). A decreasing $\log D$ correlates with an increase in solubility. Calculated and experimental values are given in columns 5 and 6 of Table 2. Oral absorption (passive diffusion) depends on the permeability of the compound and the solubility, which can be a limitation for the concentration gradient through the intestine wall. The permeability depends on lipophilicity ($\log D$), the degree of ionization and the molecular size. The factor Xa inhibitors showed a good permeability but the solubility of some derivatives was not good enough for a quantitative absorption at higher doses. Therefore, the maximum absorbable dose concept[10] (MAD) was used for the fXa inhibitors to avoid problems with the formulation of the compounds and the dosing in toxicological experiments. The target dose should be at least 10 mg/kg. To achieve this a water solubility of >300 μg/mL is necessary. This holds true for the compounds **11q**, **11r** (EMD 495235) and **11x**.

For EMD 495235 the inhibition constant (K_i value) for human Factor Xa was determined ($K_i = 6.8$ nM). EMD 495235 was also evaluated for his anticoagulant activity in the standard coagulation assay for activated thromboplastin time (APTT) and prothrombin time (PT). The concentration required to double plasma clotting time of APTT and PT was one micromolar, respectively.

Table 1. Factor Xa binding affinity data of selected chlorothiophenecarboxamides **11**



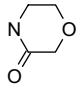
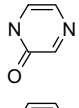
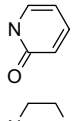
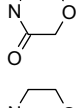
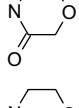
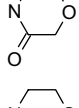
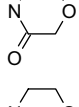
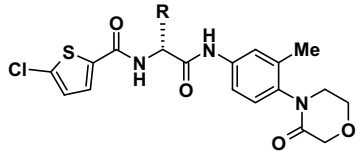
Compd	R	R^1	R^2	IC_{50} fXa (nM)
11a	H	H		700.0
11b	⋯CH_3	H		59.0
11c	⋯CH_2CH_3	H		20.0
11d	⋯$CH_2CH_2CH_3$	H		12.0
11e	⋯$CH(CH_3)_2$	H		66.0
11f	⋯$CH_2CH(CH_3)_2$	H		15.0
11g	⋯C_6H_5	H		29.0
11h	━C_6H_5	H		26.0
11i	━$CH_2CH(CH_3)_2$	H		130.0
11k	⋯$CH_2CH(CH_3)_2$	H		39.0
11l	⋯$CH_2CH(CH_3)_2$	H		16.0
11m	⋯$CH_2CH(CH_3)_2$	CH_3		7.2
11n	⋯$CH_2CH_2CH_3$	CH_3		9.5
11o	⋯CH_2CH_3	CH_3		11.0
11p	⋯CH_3	CH_3		15.0
11q	⋯CH_2OH	CH_3		29.0

Table 1 (*continued*)

Compd	R	R^1	R^2	IC_{50} fXa (nM)
11r (EMD495235)	⋯CH_2OCH_3	CH_3		5.5
11s	⋯CH_2OMe	CF_3		14.0
11t	⋯CH_2OCH_3	Cl		13.0
11u	⋯CH_2OCH_3	F		22.0
11v	⋯$CH_2OCH_2CH_3$	CH_3		10.0
11w	⋯$CH_2O(CH_2)_2CH_3$	CH_3		12.0
11x	⋯$CH_2O(CH_2)_2OCH_3$	CH_3		11.0

Table 2. Physicochemical parameters and the estimated maximum absorbable dose of selected 2-methylphenyl morpholinones



Compd	R	MW	log *D* pH 7[a]	Solubility (μg/mL)[a]	Solubility (μg/mL)[b]	MAD[c] (mg/kg)[7]
11m	$CH_2CH(CH_3)_2$	464	2.8	5	29	0.6
11w	$CH_2O(CH_2)_2CH_3$	480	1.7	37	92	1.9
11p	CH_3	422	1.4	130	134	2.8
11o	CH_2CH_3	436	1.9	41	308	6.5
11q	CH_2OH	438	1.0	260	520	11.0
11r (EMD 495235)	CH_2OCH_3	452	0.7	380	510	11.0
11x	$CH_2O(CH_2)_2OCH_3$	496	0.4	340	1054	22.0

[a] Calcd: WSKowWin 1.40 (Syracuse Res. Corp.).
[b] Meas.: shake flask, $T = 37\,^{\circ}C$, phosphate buffer pH 7.
[c] Maximum absorbable dose.

Orientating studies of the pharmacokinetics of compound EMD 495235 were investigated in Wistar rats, Beagle dogs and Cynomolgus monkeys after treatment with single intravenous and oral doses. A compilation of the pharmacokinetic parameters is presented in Table 3.

EMD 495235 is characterized by rapid absorption from the gastrointestinal tract, plasma elimination half-lives of 0.57–2.3 h, a clearance between 0.25 and 1.3 L/h/kg and absolute bioavailability of 60–80%.

EMD 495235 met our criteria so far so that in vivo investigations in different animal models were initiated. The outcome of these studies will be presented elsewhere in due course.

4. Conclusion

Based on the structural information from co-crystallization studies of different lead series chlorothiophenecarboxamides have been identified. From these investigations a promising drug candidate EMD 495235 has been characterized as a compound with good in vitro activities, acceptable water solubility and high absolute bioavailability in different animal models.

Table 3. Pharmacokinetic parameters of EMD 495235

	Rat; male ($N = 3$)		Dog; female ($N = 2$)		Monkey; female ($N = 2$)	
Administration	iv	po	iv	po	iv	po
Dose (mg/kg)	0.2	0.5	0.15	0.5	0.2	0.5
C_{max} (ng/mL)		92		161		270
t_{max} (h)		0.5		0.5		2.5
AUC (ng/mL × h)						
0–6 h		271		211		1100
0–∞	212		117		803	
$t_{1/2}$ (h)	1.1		0.57		2.3	
CL (L/h/kg)	0.94		1.3		0.25	
V_{ss} (L/kg)	1.6		0.86		0.81	
Bioavailability (%)		~60		~60		~80

EMD 495235 was chosen as a candidate for further in vivo investigations as an anticoagulating drug.

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