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NEW ANTITHROMBOTIC RGD-MIMETICS WITH HIGH BIOAVAILABILITY

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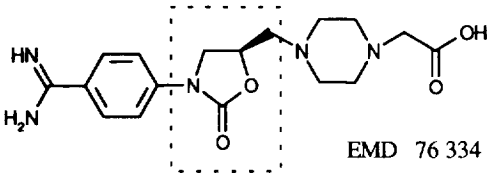
Abstract: A new class of antithrombotic RGD-mimetics with a novel oxazolidinonemethyl scaffold was synthesized. High oral activity and bioavailability was found in this series of compounds.

Introduction

We recently reported novel RGD-analogous peptidomimetics which strongly antagonize the platelet fibrinogen receptor GPIIb/IIIa[1]. Those compounds can act as highly effective and specific antithrombotics by inhibiting platelet aggregation, a potential risk factor in pathological conditions such as thrombosis, unstable angina, acute myocardial infarction, thrombotic stroke, and peripheral arterial occlusion[2].

The new system reported by us consists of a central oxazolidinonemethyl unit with basic and acidic building blocks as N- and C-terminal residues, respectively, as is common in this class of biologically active compounds[3].

As an example, EMD 76 334 (**1**), one of the most active compounds of this kind, is shown:



EMD 76 334 **1**

These novel compounds could be shown to possess strong GPIIb/IIIa-antagonistic and anti-platelet aggregation potency *in vitro*[1]. Relatively high anti-aggregatory activity in *ex vivo* experiments was found in the guinea pig after p.o. application.

However, these compounds exhibited low bioavailability (1-5 % in the guinea pig), as has been observed generally with compounds of this kind[4]. Bioavailability is, however, an important parameter for the application in humans, because low values (< ca. 20 %) can cause unacceptable interindividual fluctuations of the drug plasma level.

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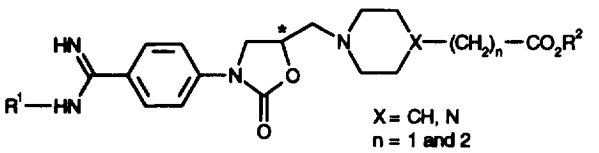
Therefore, we synthesized prodrugs of these compounds, since prodrugs have been shown to increase bioavailability in similar cases[4b,5].

Chemistry

We now report the synthesis and biological evaluation of compounds shown in the general formula **2** which are carboxylic esters with free amidino groups (R^1 = H, R^2 = alkyl) (**3**), acylamidino carboxylic acids (R^1 = acyl, R^2 = H) (**4**), and acylamidino carboxylic esters (R^1 = acyl, R^2 = alkyl) (**5**), acting as potential prodrugs or double prodrugs.

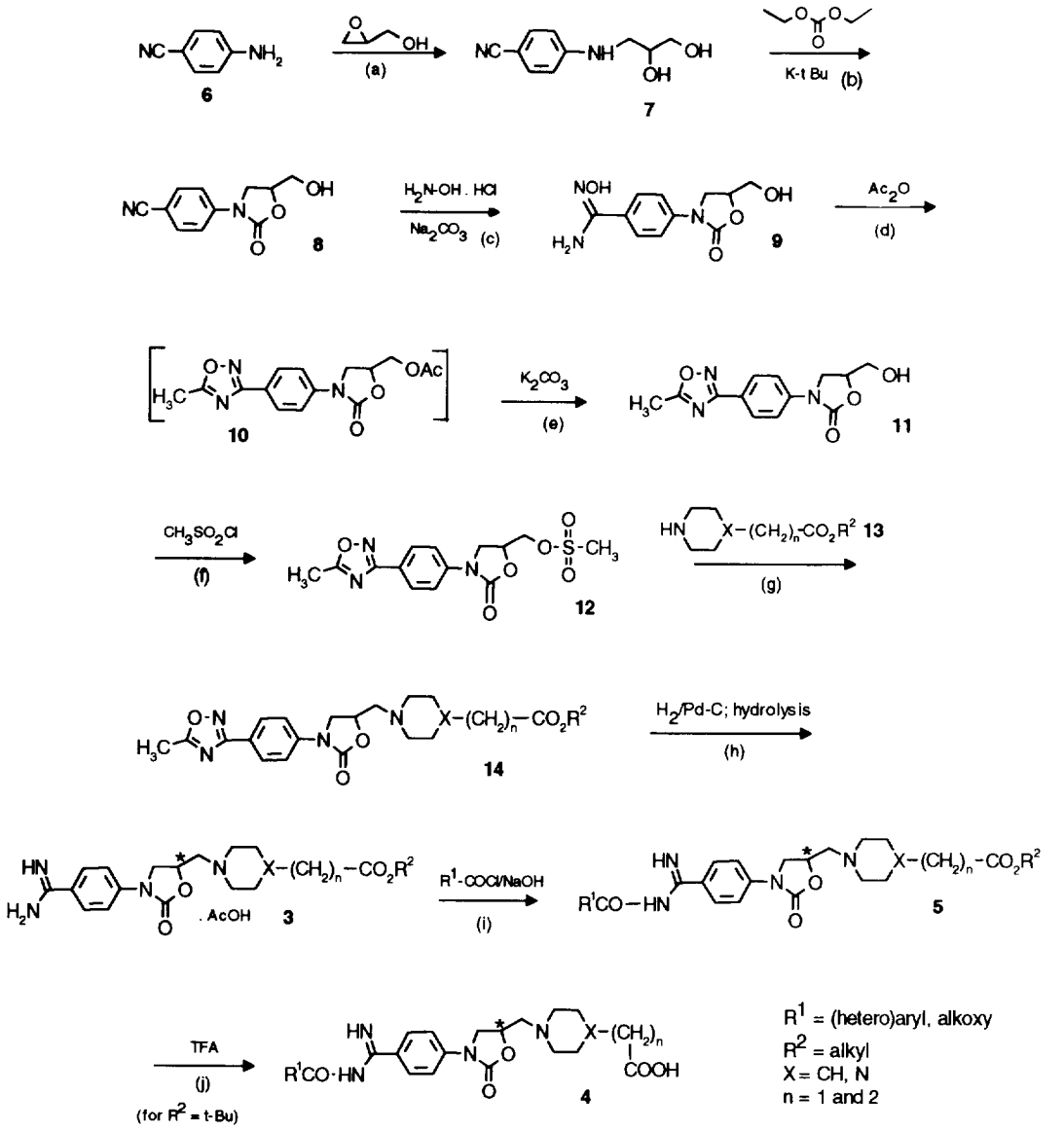


2

The synthesis of these compounds is shown in Scheme 1.

4-Aminobenzonitrile (**6**) is reacted with glycidol to give the diol **7**. This reacts with diethylcarbonate with ring closure to afford the heterocyclic alcohol **8**. Addition of hydroxylamine leads to the amidoxime **9**, the N-terminus of which is protected by cyclization with acetic anhydride furnishing the oxadiazole **10** which - without isolation - is hydrolyzed to the alcohol **11**. Mesylation of compound **11** gives the reactive building block **12** which is reacted with the piperazino acetic and propionic acid esters **13**[6] to give compounds **14**. Hydrogenation and subsequent spontaneous hydrolysis give the esters **3** with free amidino group. Acylation leads to the esters **5** which (in the case of R^2 = t-Bu) can be transformed into the acylated carboxylic acids **4** on treatment with TFA.

Scheme 1 **Synthesis of the New Oxazolidinone Peptidomimetics**



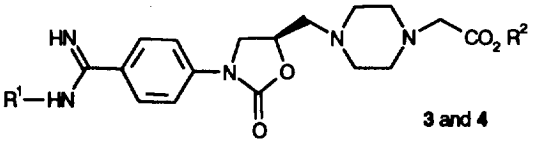
Conditions: (a) 2:1 (molar ratio), MeOH, 20 h refl., 70 % (b) 1:6:0.05, 2 h 100°C, 85 % (c) 1:3:4, MeOH, 6 h refl., 80 % (d) Ac_2O as solvent, 4 h 120°C, evaporated (e) 1:1.2, MeOH, 6 h refl., 80 % total (f) 1:1.25, pyridine, 0.5 h 5°C, 12 h r. t., 94 % (g) 1:2, acetonitrile, 16 h refl., 70-90 % (h) a) EtOH/acetic acid (2:1) b) add. of H_2O, r. t., 80-90 % (i) 1:1.2:2.2, CH_2Cl_2/H_2O, 1 h 5-8°C, 75-80 % (j) TFA as solvent, 2 h r. t., 90-95 %.

Results and Discussion

The activities of compounds **3-5** after i.v. and/or p.o. administration in the guinea pig and cynomolgus monkey and subsequent measurement of *ex vivo* collagen-induced platelet aggregation [7] are shown in tables 1 and 2. As the ED_{50} we determined that dose of compound which was able to increase the "threshold concentration" of the agonist up to the mean between the control value (mostly 2 µg/ml) and the maximal value of 16 µg/ml collagen. The given ED_{50}-values represent the maximal activity point. The "threshold value" was taken to be the lowest concentration of collagen which could produce an aggregation of 20 % width within 3 minutes, as was used by others: Loew et al. (1974)[7b] and Falchi et al. (1983)[8].

As can be seen, the potential simple prodrugs (**3** and **4**) have negligible p.o. activities in the guinea pig (table 1).

Table 1: Simple prodrugs: Ex vivo inhibition of collagen-induced platelet aggregation in the guinea pig



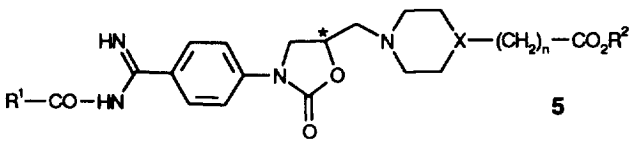
3 and 4

			ED_{50} (mg/kg)	
compound	**R^1**	**R^2**	**i. v.**	**p. o.**
3a	H	Et	0.08	> 1
3b	H	tBu	1.0	
4a	MeOCO	H		> 10
4b	benzoyl	H		> 10

In contrast, some of the double-substituted compounds **5** have considerable activities in both the guinea pig and the cynomolgus monkey (table 2). As can be seen, the urethane-type acetic acid esters **5n** and **5o** have the highest oral activities. Most of the other compounds with urethane-like N-terminus (**5p-5v**) have similar values. Most of the corresponding compounds with aroyl- or heteroaroyl N-terminal substituents (**5a - 5m**) have comparable activities. Changing from the acetic acid esters to the propionic acid esters results in a dramatic decrease of both the i.v. and p.o. activities (see **5d/5g**, and **5p/5q** for instance). In addition, the R-configured compounds in the acetic acid series are superior to those with S-configuration (see **5d/5e**) or to the racemates (see **5o/5p**).

Bioavailability was calulated from plasma levels of the active drug after p.o. application of the prodrug and i.v. application of the drug to cynomolgus monkeys. The results show **> 15 %** bioavailability in the aroyl series (best compound: **5d** = EMD 94 065) and **> 35 %** in the alkoxycarbonyl series (best compound: **5o** = EMD 122 347). The latter has been selected for further development.

Table 2: Double prodrugs: ex vivo inhibition of collagen-induced platelet aggregation in the guinea pig (g. p.) and cynomolgus monkey (c. m.)



5

						ED_{50} (mg/kg) g. p.		ED_{50} (mg/kg) c. m.	
Compd.	**R^1**	**R^2**	**X**	**n**	**config.**	**i.v.**	**p.o.**	**i.v.**	**p.o.**
5a	phenyl	Et	CH	O	(RS)		10.0		
5b	phenyl	Et	CH	1	(RS)		3.0		
5c	phenyl	Et	CH(OH)	1	(RS)		3.0		
5d	phenyl	Et	N	1	(R)	0.09	0.32	0.13	1.30
5e	phenyl	Et	N	1	(S)		> 1.0		
5f	phenyl	Et	N	2	(R)		>1.0		
5g	phenyl	Et	N	2	(S)		> 10		
5h	phenyl	tBu	N	1	(R)	0.4	1.0		> 1
5i	phenyl	tBu	N	2	(RS)		> 10		
5j	4-MeO-phenyl	Et	N	1	(R)		< 1.0		> 1
5k	3-CF_3-phenyl	Et	N	1	(R)		< 1.0		> 1
5l	3-pyridyl	Et	N	1	(RS)	1.0			
5m	2-furyl	Et	N	1	(RS)		< 1.0		> 1.0
5n	MeO	Me	N	1	(R)		0.1		0.8
5o	MeO	Et	N	1	(R)		0.2	0.07	0.5
5p	MeO	Et	N	1	(RS)	0.4	0.4		
5q	MeO	Et	N	2	(RS)		> 10		
5r	MeO	tBu	N	1	(RS)		> 1		
5s	EtO	Et	N	1	(R)		0.17		0.7
5t	BnO	Et	N	1	(R)		< 1		> 1
5u	iPr	Et	N	1	(R)		0.4		1.3
5v	phenoxy	Et	N	1	(RS)		0.2		

References and Notes

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   The number of animals was n = 3 in each test. All enantiomerically pure compounds had ee-values of >99 % which were determined chromatographically on a Chiralcel OD-H column.
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