DOI 10.1002/ardp.200400927 Arch. Pharm. Chem. Life Sci. 2005, 338, 78–86

New Oxadiazole Derivatives Showing partly Antiplatelet, Antithrombotic and Serotonin Antagonistic Properties*

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Ten new 1,2,4-oxadiazole- and six new 1,3,4-oxadiazole-carboxamides containing different lipophilic moieties (i.e. 4-biphenyl-, 1-naphthyl, phenylpropyl- and n-hexyl substituents) and additional basic groups which are mainly alkyl- and dialkylaminoalkyl residues have been synthesized and tested for antiplatelet effects in vitro (Born-test) and antithrombotic properties in vivo (laser thrombosis model). If the platelet aggregation was induced by collagen, the inhibitory effects (IC50) were between 58 μM and 300 μM. Using serotonin (5-HT) as an inducer, compound **6a** (N-(3-dimethylaminopropyl-5-(biphenyl-4-yl)-1,3,4-oxadiazole-2-carboxamide) had an IC50 = 1 μM (**12e**: (N-3-Dimethylaminopropyl)-3-(1-naphthyl)-1,2,4-oxadiazole-5-carboxamide, 6.7 μM). In an in vitro rat tail artery assay **6a** and **12e** behaved as a competitive 5-HT2A receptor antagonist (**6a**: pKB = 6.86 ± 0.04; **12e**: pKB = 6.66 ± 0.05). The antithrombotic effects of some compounds were small but significant (7−10% inhibition of thrombus formation).

Keywords: Oxadiazole derivatives; Antiaggregatory properties; 5 HT_{2A}-Antagonists

Received: July 16, 2004; Accepted: January 6, 2005 [FP927]

Introduction

An important discovery for the development of new drugs against cardiovascular diseases was the identification of the endothelium-derived relaxing factor (EDRF) [1] as nitric oxide (NO) or a labile NO-separating compound [2]. NO acts as a vasodilating agent and decreases the aggregation and adhesion of platelets. By activation of the intracellular located soluble guanylyl cyclase (sGC) NO causes an increase in the concentration of cyclic guanosine monophosphate (cGMP). The reference compound 3-(5-hydroxymethyl-2 furyl)-1 phenylmethyl-benzo[c]pyrazole (YC 1) directly activates the enzymes sGC [3] in a NO-independent manner whereupon the level of cGMP increases.

Simultaneously, YC 1 inhibits several isoforms of phosphodiesterases (PDE) [4]. These enzymes catalyze the decomposition of cGMP by hydrolysis.

The aim of the present study was to synthesize new compounds with antiaggregatory and antithrombotic activity by stimulating the sGC and inhibiting those types of phosphodiesterases which are found in blood platelets (see Figure 1).

Based on the findings of Straub et al. [5] who showed that the introduction of basic moieties in analogues of YC 1 increased the pharmacological effects in vitro and in vivo we wonder whether this is also true for non condensed heterocycles such as oxadiazoles.

Chemistry

1,3,4-Oxadiazole-2-carboxamides **6** were prepared according to Figure 2. The synthesis of oxalic acid ethyl ester hydrazide **3** from hydrazine hydrate **1** and oxalic acid diethyl

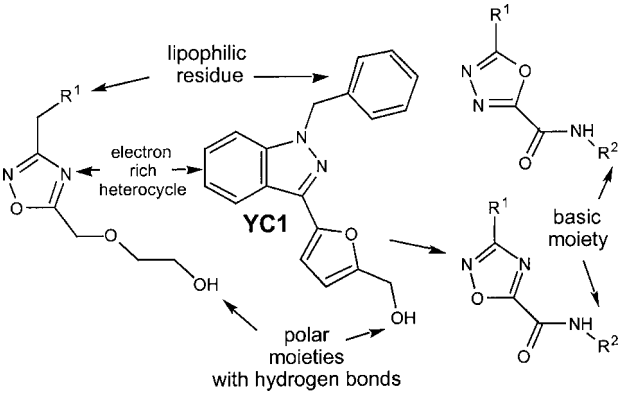

Figure 1. 1,2,4-Oxadiazoles and 1,3,4-oxadiazoles derived from YC-1.

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* Part of the PhD thesis Katrin Bethge, FU Berlin, 2002.

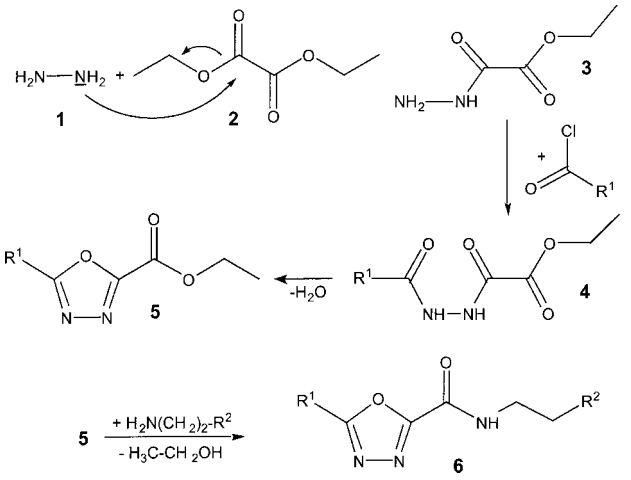

Figure 2. Synthesis of 1,3,4-oxadiazole-2-carboxamides 6 from hydrazine 1 and oxalic acid diethyl ester 2.

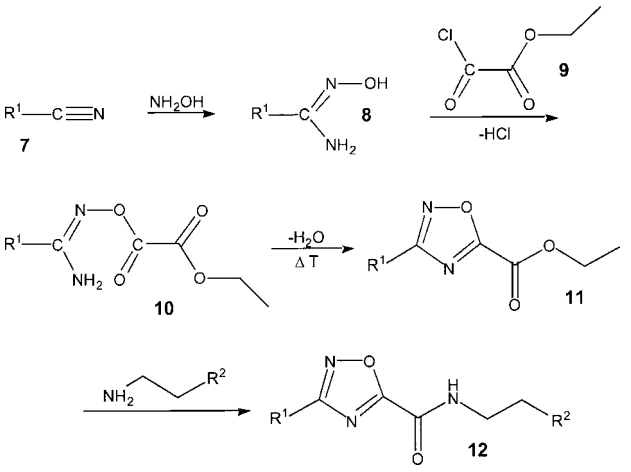

Figure 3. Synthesis of 1,2,4-oxadiazole-5-carboxamides **12** from the nitriles **7** and hydroxylamine.

ester **2** which was first described by Stollé [6]. The acylation with acid chlorides to N'-acyloxalic acid ethyl ester hydrazide **4** was carried out in dry tertahydrofuran [7]. Heating with $POCl_3$ led to the 1,3,4 oxadiazole-2-carboxylicacid-ethylesters **5**. The final step to the resulting 1,3,4 oxadiazole-2-carboxamides **6** was the reaction with various amines.

The isomeric 1,2,4-oxadiazoles were prepared as according to Figure 3. Two equivalents of hydroxyl amine were added to the nitrile in order to get the carboxamidoximes **8** (Knudsen [8]). The second step was the reaction with oxalic acid ethyl ester chloride **9** according to the procedure of Palazzo [11]. Some compounds of type 10 cyclized spontaneously by elimination of water. Other compounds were boiled in toluene for 2 h in order to obtain the 1,2,4-oxadiazole-5-carboxylicacidethylesters **11**. Subsequent reaction with amines led to a number of 1,2,4-oxadiazole-5 carboxamides **12**.

Results and discussion

Inhibition of blood platelet aggregation in vitro (Born-test)

The platelet aggregation experiments were carried out as described by Born [12] and Seuter [13]. In brief, platelet rich plasma (PRP) was prepared from freshly drawn citrated human blood by mild centrifugation. The platelet aggregation was normally induced by collagen. The time-dependent increase in light transmission was recorded in an APACT aggregometer (control).

The PRP was incubated with different concentrations of the test compound. The influence of collagen on the light transmission was recorded again. The concentration which inhibited the platelet aggregation half-maximally (IC_{50}) was determined graphically from the percentage of inhibition at various concentrations (lg c) of the test compound. The results are summarized in Table 1.

The table contains 1,3,4-oxadiazoles (**6a–j**) and 1,2,4-oxadiazoles (**12a–f**). In the row of the type 6 compounds the lipophilic biphenyl rest R^1 which already had proven to be very suitable for antiplatelet activities [9, 10] was held constant (**6a–g**, **6j**) and R^2 was varied. Especially substituents with a basic moiety (pK_B ~5) were chosen according to former experiences with ether heterocycles [9, 10]. The exception is **6f** with neutral R^2 (i.e. $-CH_2OH$) which gave not unexpectedly an inactive compound. The other rests are secondary (**6d**, **6e**, **6g**) or tertiary (**6a**, **6b**, **6c**, **6j**) amines which all exhibit antiaggregatory activity when the aggregation is induced by collagen. Comparison of **6a** and **6e** shows only a small difference between secondary and tertiary amines (IC_{50} 65 vs. 84 μM). Dimethylamino- and diethylamino functions give comparable results (IC_{50} **6a** = 65 vs. **6c** = 93 μM). A shorter chain length in R^2 is not favorable (IC_{50} **6a** = 65 vs. **6b** = 170 μM). A hydroxy group at the end of R^2 similar to YC 1 (see **6d**) is neither essential nor favorable. Cyclic amines like 1-methylpyrrolidine-2-yl (see **6j**, IC_{50} = 86 μM) are acceptable. The best R^2 i.e. dimethylaminomethyl (**6a**) was chosen for comparison with other R^1 rests (**6a** vs. **6h** or **6i**). Small activity (**6h**, IC_{50} = 230 μM, R^1 = hexyl) or total lack of activity (**6i** IC_{50} >300 μM, R^1 = 3-phenylpropyl) is observed.

The type **12** 1,2,4-oxadiazole derivatives show a pattern of activities very similar to the type 6 compounds e.g. IC_{50} **6a** = 65 vs. **12a** = 84 μM, **6b** = 170 vs. **12b** = 125 μM, **6d** = 160 vs. **12d** = 105 μM and **12f** = 58 vs. **6e** = 84 M. Concerning R^1 a naphthyl substituent is nearly equipotent to a biphenyl rest (see **12a** vs. **12e**). The most active **12f** (IC_{50} = 58 μM) is comparable to **6e** (IC_{50} = 84 μM).

To get more information on the mechanism of the antiplatelet activity three 1,3,4-oxadiazoles (**6a**, **6d**, **6e**) and two 1,2,4-oxadiazoles (**12e**, **12f**) were selected for an investigation in the Born-test using other inducers than collagen

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Table 1. The in vitro antiplatelet properties of oxadiazoles. In the Born-test the relative standard deviation is <10% see asa. The other assay were run in duplicate.

Compound	R^1	R^2	Born test IC_{50} [μM]
6a	4-Biphenyl-	$-CH_2-N(CH_3)_2$	65
6b	4-Biphenyl-	$-N(CH_3)_2$	170
6c	4-Biphenyl-	$-CH_2-N(CH_2-CH_3)_2$	93
6d	4-Biphenyl-	$-CH_2-NH(CH_2)_2OH$	160
6e	4-Biphenyl-	$-CH_2-NHCH_3 \cdot HCl$	84
6f	4-Biphenyl-	$-CH_2-OH$	>300
6g	4-Biphenyl-	$-NH(CH_2)_2OH$	270
6h	C_6H_{13}-	$-CH_2-N(CH_3)_2$	230
6i	Phenyl-$(CH_2)_3$-	$-CH_2-N(CH_3)_2$	>300
6j	4-Biphenyl-	1-Methylpyrrolidine-2-yl-	86
12a	4-Biphenyl-	$-CH_2-N(CH_3)_2$	84
12b	4-Biphenyl-	$-N(CH_3)_2$	215
12c	4-Biphenyl-	$-CH_2-N(CH_2-CH_3)_2$	190
12d	4-Biphenyl-	$-CH_2-NH(CH_2)_2OH$	105
12e	1-Naphthyl-	$-CH_2-N(CH_3)_2$	93
12f	4-Biphenyl-	$-CH_2-NHCH_3 \cdot HCl$	58
asa	–	–	175 ± 20

Table 2. IC_{50} values for 1,2,4- and 1,3,4-oxadiazole-carboxamides using different inducers of aggregation in the Born test. The relative standard deviation is <10%.

	IC_{50} of the test compounds [μM]				
Inducer of aggregation	**6a**	**6d**	**6e**	**12e**	**12f**
Collagen	65	160	84	93	58
ADP	118	60	53	105	63
5-HT	1.0	110	75	6.7	27
Adrenaline	110	215	> 300	217	160
PAF	28	52	120	28	150

for the platelet aggregation. The criterium for selection was the ability to inhibit the aggregation by collagen. For comparison one compound (**6d**) with small activity was included.

Table 2 shows the results of the Born test with other inducers of the platelet aggregation. The IC_{50} values of standard drugs were: WEB 2086 (apafant) 2 μM; phentolamine mesylate 3 μM; NECA, 5′-N-ethylcarboxamidoadenosine 2 μM; imipramine hydrochloride 5 μM.

It is evident that the platelet aggregation induced by serotonin can be inhibited by rather small concentrations of some compounds. The best effects were observed with compounds **12e** (IC_{50} ~7 μM) and **6a** (IC_{50} ~1 μM). The induction by ADP, adrenaline and PAF is inhibited in the same order of magnitude as by collagen. Therefore, it is suggested that the mechanism of action at least in **6a** and **12e** is associated with 5-HT.

Inhibition of thrombus formation in vivo (laser-thrombosis model)

In order to examine the inhibition of thrombosis of our oxadiazoles we used an *in vivo* thrombosis model [14]. In brief, the formation of thrombi in mesenteric vessels of rats was induced by the beam of an argon laser via a microscope (35 mW, 50 ms). The number of exposures ("shots") necessary to form a thrombus of defined size was counted. From the average shot number the percentage of inhibition of thrombosis was calculated.

All compounds of Table 1 were tested but only **6b**, **6d**, **6e** showed small antithrombotic effects. The effect of **12d** has to be regarded as marginal (see Table 3). The common structural feature obviously is a 4-biphenyl rest as R^1 and a basic moiety with pK_B ~5 in R^2. However several of other compounds of Table 1 do fulfill these structural requirements without showing antithrombotic activity. Therefore

Table 3. Antithrombotic effects of oxadiazoles 2 h after p.o. administration of 60 mg/kg of the test compound to rats. Statistics: Wilcoxon Man Whitney U test, n.s. – not significant.

Compound	R^1	R^2	Inhibition of thrombus formation in venules		arterioles		IC_{50}
			% ± s_x	p ≤	% ± s_x	p ≤	µmol/L
6b	4-Biphenyl-	$-N(CH_3)_2$	2 ± 1	n.s.	7 ± 1	0.01	170
6d	4-Biphenyl-	$-CH_2-NH(CH_2)_2OH$	0 ± 2	n.s.	7 ± 1	0.01	160
6e	4-Biphenyl-	$-CH_2-NHCH_3 \cdot HCl$	4 ± 1	0.02	10 ± 1	0.002	84
12d	4-Biphenyl-	$-CH_2-NH(CH_2)_2OH$	0 ± 1	n.s.	4 ± 1	0.1	105
asa	–	–	20 ± 5	0.002	48 ± 10	0,002	175

concise structure activity relationships cannot be drawn. Comparison of **6d** with **12d** which only differ in the oxadizole part shows a slight advantage for the 1,3,4-oxadiazole structure. Derivative **6e** had the highest antithrombotic effect in this model.

Activation of sGC

Compounds **6a**, **6d**, **6e**, **12e** and **12f** were tested by the method of Hoenicka et al. [15] in a heme-containing sGC-assay [14] in the presence of Mg^{2+} ions towards their ability to stimulate sGC (Institute of Cardiovascular Research, Bayer AG, Wuppertal).

There was no increase of the basal activity of sGC measured with any of the compounds tested. Even the addition of DEA/NO (N,N-dimethylamino-diazenolat-2-oxide) did not show an additional effect as described for YC-1 and the reference substance BAY 41-8543, respectively [16, 17].

Inhibition of phosphodiesterases

In order to examine the effect towards several isoforms of phosphodiesterases, compounds **6a**, **6d**, **6e**, **12e** and **12f** were tested for their inhibitory activity in different cGMP-SPA [15] and cAMP-SPA tests (Amersham Biosciences) at the Institute of Cardiovascular Research, Bayer AG, Wuppertal.

No inhibition was observed (IC_{50} >100 µM) against PDE 1, 2, 3, 4, 5, or 9 for any of the oxadiazole derivatives tested.

Antagonism at the 5-HT_{2A}-receptor

Since 5-HT_{2A} receptors are involved in 5-HT-induced platelet aggregation, we tested the inhibition of the compounds **6a** and **12e** against the contractile effect of 5-HT in the isolated rat tail artery [18] which is a tissue that is endowed with 5-HT_{2A} receptors. Details about experimental procedure are given in reference [18]. Compound **6a** concentration-dependently antagonized the contractile response to 5-HT. Concentration-response curves to 5-HT were shifted to the right in a parallel manner (Figure 4). The Schild analysis [19] gave a pK_B value of 6.86 ± 0.03 (n = 12). The slope of the Schild plot was 0.90 ± 0.05 and not significantly different from unity (p <0.05) [20]. Compound **12e** showed a nearly identical behavior. The pK_B value is 6.66 ± 0.06. The slope of the Schild is plot 1.06 ± 0.08 and as well not significantly different from unity (p <0.05). In this model

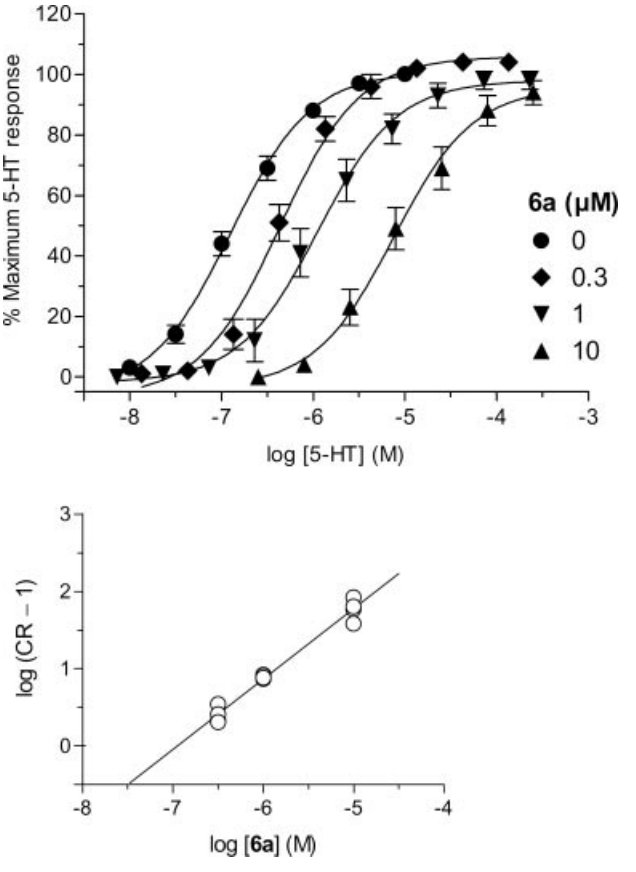

Figure 4. Antagonism of 5-HT-induced contraction by **6a** in rat tail artery. The upper panel represents cumulative concentration-response curves to 5-HT in the absence and presence of **6a**. The data are mean ± SEM from 4 experiments each. The lower panel represents the Schild regression analysis.

the standard drug ketanserin showed a $pK_B = 9.55 \pm 0.02$. Sarpogrelate gave a $pK_B = 8.46 \pm 0.04$. We conclude that **6a** and **12e** act as a competitive antagonist of 5-HT at the $5\text{-}HT_{2A}$ receptor of rat tail artery. In summary, the results indicate that the effects of compound **6a** and **12e** in the Born test are related to a competitive antagonism against 5-HT at the $5\text{-}HT_{2A}$ receptor.

Experimental

Chemistry

Mp. (uncorr.), Linström. Elemental analysis: Elementar Vario EL. IR: Perkin Elmer 1420 Ratio Recording IR-Spectrophotometer and ATI Mattson Genesis Serie FTIR. NMR: Bruker Avance/DPX 400. EI-MS: CH-7A-Varian MAT (70 eV). The synthesis of **3** [6] and **8b** [19] already has been reported. All other compounds were prepared for the first time.

*Synthesis of oxalic acid ethyl ester hydrazide **3** (method of Stollé [6])*

To 16.6 g oxalic acid ethyl ester (0.11 mol) dissolved in 6 mL ethanol was dropped slowly the equivalent amount of hydrazine hydrate (5.7 g) in ethanol at a temperature of -10 to -15 °C. Subsequently, the solvent was removed in vacuo at temperatures below 25 °C.

Powder, mp. 71 °C (ethanol), yield 13 g (87%). Anal. $C_4H_8N_2O_3$ (132.2). ^{1}H-NMR / 400 MHz ([D$_6$]DMSO): δ (ppm) = 1.25 (t, J = 7.1 Hz, 3H, CH_3), 4.21 (q, J = 7.1 Hz, 2H, CH_2), 9.92 (s, 2H, NH_2, D_2O exchange), 10.21 (s, 1H, NH, D_2O exchange). MS (EI, 80 °C): m/z (%) = 132 (29) [$M^{+\bullet}$], 104 (65) [$M^{+\bullet}$-C_2H_4], 76 (15), 59 (41), 58 (68), 45 (43), 43 (10), 32 (51), 31 (100) [$N_2H_3^+$], 30 (23).

*General procedure for the synthesis of N^2-acyloxalic acid ethyl ester hydrazides (**4**) (method of Dost et al. [7] slightly modified)*

Dry oxalic acid ethyl ester hydrazide 3 (0.1 mol) was suspended in 25 mL of dry tetrahydrofuran and mixed with 0.1 mol $NaHCO_3$. Then 0.1 mol acid chloride was dropped into the suspension within 30 min at a temperature of 45 °C. The acid chlorides were obtained from the corresponding acids by boiling with freshly distilled thionyl chloride followed by fractionated distillation. The suspension was stirred for 12 h at 45–50 °C. The access of water was carefully avoided. Then the mixture was filtered and the solvent removed in vacuo. The products were recrystallized from ether. It was not possible to obtain the compounds analytically pure because of their insolubility in organic solvents and their tendency to decompose or to undergo cyclization during heating.

*Oxalic acid ethyl ester-N^2-biphenyl-4-yl-carbonylhydrazide (**4a**)*

From 6.0 g (0.03 mol) 4-biphenyl-carboxylic acid chloride. Powder, mp. 108 °C, yield 5.9 g (41%). Anal. $C_{17}H_{16}N_2O_4$ (312.2). ^{1}H-NMR / 400 MHz ([D$_6$]DMSO): δ (ppm) = 1.32 (t, J = 7.2 Hz, 3H, CH_3), 4.31 (q, J = 7.2 Hz, 2H, CH_2-CH_3), 7.43 (t, J = 7.2 Hz, 1H, biph 4′-H), 7.41–7.44 (m, 2H, biph 3′-H, 5′-H), 7.75 (d, J = 7.8 Hz, 2H, biph 2′-H, 6′-H), 7.83 (d, J = 8.1 Hz, 2H, biph 2-H, 6-H), 7.98 (d, J = 8.2 Hz, 2H, biph 3-H, 5-H), 10.63 (s, 1H, NH-(C=O)Ph, D_2O exchange), 10.95 (s, 1H, NH-(C=O)C=O, D_2O exchange). MS (EI, 130 °C): m/z (%) = 312 (3) [$M^{+\bullet}$], 182 (14), 181 (100), 153 (16), 152 (23).

*Oxalic acid ethyl ester- N^2-hexylcarbonylhydrazide (**4b**)*

From 1.5 g (10 mmol) hexane-carboxylic acid chloride. Powder, mp. 52 °C, yield 0.3 g (13%). Anal. $C_{11}H_{20}N_2O_4$ (244.2). ^{1}H-NMR / 400 MHz ([D$_6$]DMSO): δ (ppm) = 0.85–0.88 (m, 3H, CH_3-$(CH_2)_5$), 1.19–1.32 (m, 9H, CH_3-CH_2O and $(CH_2)_3$-CH_3), 1.47–1.53 (m, 2H, CH_2-$(CH_2)_3CH_3$), 2.13 (t, J = 7.3 Hz, 2H, CH_2C=O), 4.26 (q, J = 7.1 Hz, 2H, OCH_2), 9.91 (s, 1H, NH-(C=O)CH_2, D_2O exchange), 10.66 (s, 1H, NH-(C=O)C=O, D_2O exchange). MS (EI, 100 °C): m/z (%) = 244 (2) [$M^{+\bullet}$], 171 (13), 132 (18), 113 (81) [$C_7H_{13}O^+$], 85 (30), 57 (15), 55 (12), 43 (100) [$C_3H_7^+$], 41 (19).

*Oxalic acid ethyl ester-N^2-(3-phenylpropyl)-carbonylhydrazide (**4c**)*

From 16.4 g (0.1 mol) 4-phenylbutyricacid. Powder, mp. 51 °C, yield 7.6 g (27%). Anal. $C_{14}H_{18}N_2O_4$ (278.2). ^{1}H-NMR / 400 MHz ([D$_6$]DMSO): δ (ppm) = 1.28 (t, J = 7.0 Hz, 3H, CH_3), 1.77–1.84 (m, 2H, CH_2-CH_2Ph), 2.21 (t, J = 7.5 Hz, 2H, CH_2C=O), 2.60 (t, J = 7.5 Hz, 2H, CH_2-Ph), 4.26 (q, J = 7.1 Hz, 2H, CH_2-CH_3), 7.16–7.30 (m, 5H, Ph), 9.96 (s, 1H, NH-(C=O)CH_2, D_2O exchange), 10.69 (s, 1H, NH-(C=O)C=O, D_2O exchange). MS (EI, 110 °C): m/z (%) = 278 (6) [$M^{+\bullet}$], 220 (15), 174 (43), 147 (77), 132 (22), 129 (13), 105 (10), 104 (25), 91 (100) [$C_7H_7^+$], 74 (25), 65 (11), 41 (13), 32 (14).

*General procedure for the cyclization of the ester acylhydrazides **4** to 1,3,4-oxadiazole-2-carboxylic acid ethyl esters **5** (method from Dost et al. [7] modified)*

0.02 mol of the corresponding oxalicacidethylester-N^2-acylhydrazide were stirred with 30 mL $POCl_3$ for 5 h at 65 °C. The excess of $POCl_3$ was removed in vacuo (2000 Pa) at temperatures below 40 °C. The residue was poured carefully on ice. The mixture was adjusted to pH = 6.5–7.0 with 10% NaOH and extracted with ether several times. The combined amounts of ether were dried and the solvent was removed in vacuo. The final purification was performed by recrystallization in the solvent stated or by column chromatography.

*5-(Biphenyl-4-yl)-1,3,4-oxadiazole-2-carboxylic acid ethyl ester (**5a**)*

From 5.9 g (0.02 mol) **4a**. Crystals, mp. 102 °C (ether), yield 4.7 g (84%). Anal. $C_{17}H_{14}N_2O_3$ (294.3). IR (KBr): ν = 1740 cm^{-1} (C=O); 1610 (C=N); 1570 ^{1}H-NMR / 400 MHz ([D$_6$]DMSO): δ (ppm) = 1.39 (t, J = 7.1 Hz, 3H, CH_3), 4.47 (q, J = 7.1 Hz, 2H, CH_2), 7.44–7.47 (m, 1H, biph 4′-H), 7.52–7.55 (m, 2H, biph 3′-H, 5′-H), 7.79 (d, J = 7.4 Hz, 2H, biph 2′-H, 6′-H), 7.96 (d, J = 8.4 Hz, 2H, biph 2-H, 6-H), 8.16 (d, J = 8.3 Hz, 2H, biph 3-H, 5-H). MS (EI, 120 °C): m/z (%) = 295 (16), 294 (88) [$M^{+\bullet}$], 221 (36) [M^+-$C_3H_5O_2$], 181 (41), 180 (16), 179 (100), 153 (15), 152 (20).

*5-Hexyl-1,3,4-oxadiazole-2-carboxylic acid ethyl ester (**5b**)*

From 4.9 g (0.02 mol) **4b**. Oil, Eluent chloroform, yield 3.1 g (68%). Anal. $C_{11}H_{18}N_2O_3$ (226.3). IR (film): ν = 1749 cm^{-1} (C=O); 1556 (C=N). ^{1}H-NMR / 400 MHz ([D$_6$]DMSO): δ (ppm) = 0.86 (t, J = 7.0 Hz, 3H, CH_3-$(CH_2)_5$), 1.26–1.29 (m, 6H, $(CH_2)_3$-CH_3), 1.33 (t, J = 7.1 Hz, 3H, CH_3-CH_2O), 1.68–1.74 (m, 2H, CH_2-CH_2Ox), 2.94 (t, J = 7.5 Hz, 2H, CH_2Ox), 4.41 (q, J = 7.1 Hz, 2H, OCH_2-CH_3). MS (EI, 80 °C): m/z (%) = 226 (2) [$M^{+\bullet}$], 197 (10), 183 (20), 169 (44), 156 (100) [M^+-C_5H_{10}], 153 (32) [M^+-$C_3H_5O_2$], 128 (17), 113 (20), 69 (13), 55 (18), 43 (48) [$C_3H_7^+$], 41 (36).

*5-(3-Phenylpropyl)-1,3,4-oxadiazole-2-carboxylic acid ethyl ester (**5c**)*

From 8.1 g (0.03 mol) **4c**. Oil, Eluent dichloromethane/methanol (99:1), yield 1.0 g (13%). Anal. $C_{14}H_{16}N_2O_3$ (260.3). IR (film): ν =

1747 cm^{-1} (C=O); 1553 (C=N). ^{1}H-NMR / 400 MHz ([D_6]DMSO): δ (ppm) = 1.33 (t, *J* = 7.1 Hz, 3H, CH_3-CH_2O), 1.99−2.07 (m, 2H, CH_2-CH_2Ph), 2.68 (t, *J* = 7.6 Hz, 2H, CH_2Ph), 2.94 (t, *J* = 7.4 Hz, 2H, CH_2Ox), 4.41 (q, *J* = 7.1 Hz, 2H, OCH_2-CH_3), 7.17−7.31 (m, 5H, Ph). MS (EI, 90 °C): m/z (%) = 260 (4) [$M^{+\bullet}$], 156 (100) [M^+-C_8H_8], 128 (20), 91 (19) [$C_7H_7^+$].

General procedure for the synthesis of the 1,3,4-oxadiazole-2-carboxamides ***6*** *(method from Dost et al. [7] modified)*

Method A: 0.02 mol of the corresponding ester were dissolved in 50 mL methanol or ethanol, mixed with 0.02 mol of the amine and refluxed for 5 min. After cooling, solid crystals were isolated. Further product was obtained by concentrating the solution. The final cleaning was achieved by recrystallization from methanol/water.

Method B: One equivalent of the corresponding ester was dissolved in dichloromethane and mixed with 1.5 equivalents of the amine. The solution was heated under reflux for 30 min. The solvent was removed in vacuo and the residue recrystallized from methanol.

Method C: One equivalent of the corresponding ester was dissolved in dichloromethane. Two equivalents of the amine were dropped into the solution. After stirring and carefully avoiding the access of oxygen for 1−2 wk at room temperature the solvent was removed in vacuo. The residue was dissolved again in dichloromethane, washed with water until neutral reaction was reached, and then mixed three times with 0.1 N-HCl. The combined volumes of hydrochloric acid were mixed with a saturated solution of Na_2CO_3 until basic reaction occurred. Subsequently, the solution was extracted with dichloromethane. After drying the solution with Na_2SO_4 the solvent was removed in vacuo. A final cleaning step was achieved by column chromatography or recrystallisation.

*N-(3-Dimethylaminopropyl)-5-(biphenyl-4-yl)-1,3,4-oxadiazole-2-carboxamide (****6a****)*

From 840 mg (2.9 mmol) **5a** (method C). Powder, mp. 104 °C (ether), yield 620 mg (62%). Anal. $C_{20}H_{22}N_4O_2$ (350.4). IR (KBr): ν = 1691 cm^{-1} (C=O); 1613 (C=N). ^{1}H-NMR/400 MHz ([D_6]DMSO): δ (ppm) = 1.66−1.73 (m, 2H, CH_2-CH_2N), 2.15 (s, 6H, $(CH_3)_2N$), 2.28 (t, *J* = 7.0 Hz, 2H, CH_2-$N(CH_3)_2$), 3.32 (after D_2O exchange t, *J* =7.1 Hz, 2H, CH_2-NHCO), 7.45 (t, *J* = 7.3 Hz, 1H, biph 4′-H), 7.51−7.55 (m, 2H, biph 3′-H, 5′-H), 7.78−7.81 (m, 2H, biph 2′-H, 6′-H), 7.96 (d, *J* = 8.4 Hz, 2H, biph 2-H, 6-H), 8.16 (d, *J* = 8.4 Hz, 2H, biph 3-H, 5-H), 9.42 (t, *J* =5.7 Hz, 1H, NH, D_2O exchange). MS (EI, 100 °C): m/z (%) = 308 (<1) [$M^{+\bullet}$], 58 (100) [$C_3H_8N^+$].

*N-(2-Dimethylaminoethyl)-5-(biphenyl-4-yl)-1,3,4-oxadiazole-2-carboxamide (****6b****)*

From 730 mg (2.5 mmol) **5a** (method C). Powder, mp. 110 °C (methanol), yield 340 mg (42%). Anal. $C_{19}H_{20}N_4O_2$ (336.4). IR (KBr): ν = 1698 cm^{-1} (C=O); 1613 (C=N). ^{1}H-NMR/400 MHz ([D_6]DMSO): δ (ppm) = 2.19 (s, 6H, $(CH_3)_2N$), 2.44 (t, *J* = 6.7 Hz, 2H, CH_2-$N(CH_3)_2$), 3.38−3.44 (m, 2H, CH_2-NHCO), 7.43−7.47 (m, 1H, biph 4′-H), 7.51−7.55 (m, 2H, biph 3′-H, 5′-H), 7.79 (d, *J* = 7.4 Hz, 2H, biph 2′-H, 6′-H), 7.96 (d, *J* = 8.4 Hz, 2H, biph 2-H, 6-H), 8.17 (d, *J* = 8.3 Hz, 2H, biph 3-H, 5-H), 9.18−9.19 (m, 1H, NH, D_2O exchange.). MS (EI, 110 °C): m/z (%) = 336 (<1) [$M^{+\bullet}$], 58 (100) [$C_3H_8N^+$].

*N-(3-Diethylaminopropyl)-5-(biphenyl-4-yl)-1,3,4-oxadiazole-2-carboxamide (****6c****)*

From 530 mg (1.8 mmol) **5a** (method C). Crystals, mp. 70.5 °C (methanol), yield 290 mg (35%). Anal. $C_{22}H_{26}N_4O_2$ (378.5). IR (KBr): ν = 1687 cm^{-1} (C=O); 1611 (C=N). ^{1}H-NMR/400 MHz ([D_6]DMSO): δ (ppm) = 0.97 (t, *J* = 7.1 Hz, 6H, 2x CH_3), 1.65−1.72 (m, 2H, CH_2-CH_2N), 2.44−2.48 (m, 6H, 3 x CH_2N), 3.36 (after D_2O exchange t, *J* = 6.9 Hz, 2H, CH_2-NHCO), 7.45 (t, *J* = 7.3 Hz, 1H, biph 4′-H), 7.51−7.55 (m, 2H, biph 3′-H, 5′-H), 7.79 (d, *J* = 7.7 Hz, 2H, biph 2′-H, 6′-H), 7.96 (d, *J* = 8.4 Hz, 2H, biph 2-H, 6-H), 8.16 (d, *J* = 8.4 Hz, 2H, biph 3-H, 5-H), 9.48 (t, *J* =5.4 Hz, 1H, NH, D_2O exchange.). MS (EI, 90 °C): m/z (%) = 378 (3) [$M^{+\bullet}$], 86 (100) [$C_5H_{12}N^+$], 72 (15) [$C_4H_{10}N^+$].

*N-[3-(2-Hydroxyethylamino)-propyl]-5-(biphenyl-4-yl)-1,3,4-oxadiazole-2-carboxamide (****6d****)*

From 540 mg (1.8 mmol) **5a** (method C). Powder, mp. 135 °C (methanol), yield 430 mg (63%). Anal. $C_{20}H_{22}N_4O_3$ (366.4). IR (KBr): ν = 1688 cm^{-1} (C=O); 1611 (C=N). ^{1}H-NMR/400 MHz ([D_6]DMSO): δ (ppm) = 1.66−1.73 (m, 2H, CH_2-CH_2-CH_2), 2.56−2.62 (m, 4H, CH_2-NH-CH_2), 3.35−3.38 (m, after D_2O exchange t, *J* = 6.8 Hz, 2H, CH_2-NHCO), 3.45 ("q", *J* = 5.5 Hz, 2H, CH_2-OH), 4.44 (t, *J* = 5.3 Hz, 1H, OH, D_2O exchange), 7.43−7.47 (m, 1H, biph 4′-H), 7.51-7.55 (m, 2H, biph 3′-H, 5′-H), 7.78−7.80 (m, 2H, biph 2′-H, 6′-H), 7.95 (d, *J* = 8.4 Hz, 2H, biph 2-H, 6-H), 8.17 (d, *J* = 8.3 Hz, 2H, biph 3-H, 5-H), 9.46 (br. s, 1H, NHCO, D_2O exchange). MS (EI, 180 °C): m/z (%) = 366 (3) [$M^{+\bullet}$], 336 (25), 335 (100) [$M^{+\bullet}$-CH_3O], 306 (17) [$M^{+\bullet}$-C_2H_6NO], 223 (34), 181 (23), 179 (33), 153 (10), 152 (12), 113 (18), 74 (12) [$C_3H_8NO^+$], 56 (13), 44 (26).

*N-(3-Methylaminopropyl)-5-(biphenyl-4-yl)-1,3,4-oxadiazole-2-carboxamide hydrochloride (****6e****)*

From 580 mg (2.0 mmol) **5a** (method B). Crystals, mp. 198 °C (methanol/HCl), yield 290 mg (39%). Anal. $C_{19}H_{21}ClN_4O_2$ (372.8). IR (KBr): ν = 2441 cm^{-1} (NH_2^+), 1693 (C=O); 1612 (C=N). ^{1}H-NMR / 400 MHz ([D_6]DMSO): δ (ppm) = 1.86−1.93 (m, 2H, CH_2-CH_2N), 2.56 (t, *J* = 5.4 Hz, 3H, CH_3N), 2.92−2.99 (m, 2H, CH_2N^+), 3.40-3.46 (m, 2H, CH_2-NHCO), 7.44−7.48 (m, 1H, biph 4′-H), 7.52−7.56 (m, 2H, biph 3′-H, 5′-H), 7.79−7.81 (m, 2H, biph 2′-H, 6′-H), 7.97 (d, *J* = 8.4 Hz, 2H, biph 2-H, 6-H), 8.17−8.19 (m, 2H, biph 3-H, 5-H), 8.63 (br. s, 2H, NH_2^+, D_2O exchange), 9.51 (t, *J* = 5.9 Hz, 1H, NHCO). MS (EI, 80 °C): m/z (%) = 336 (4) [$M^{+\bullet}$ base], 236 (11), 181 (10), 179 (19), 71 (30), 70 (19), 58 (18) [$C_3H_8N^+$], 44 (100) [$C_2H_6N^+$], 36 (15), 28 (15).

*N-(3-Hydroxypropyl)-5-(biphenyl-4-yl)-1,3,4-oxadiazole-2-carboxamide (****6f****)*

From 910 mg (3.1 mmol) **5a** (method A). Powder, mp. 166 °C (ethanol), yield 570 mg (57%). Anal. $C_{18}H_{17}N_3O_3$ (323.4). IR (KBr): ν = 1687 cm^{-1} (C=O); 1612 (C=N). ^{1}H-NMR / 400 MHz ([D_6]DMSO): δ (ppm) = 1.69−1.76 (m, 2H, CH_2-CH_2OH), 3.35−3.40 (m, 2H, CH_2-NHCO), 3.47-3.51 (m, 2H, CH_2-OH), 4.53 (t, *J* = 5.1 Hz, 1H, OH, D_2O exchange), 7.45 (t, *J* = 7.3 Hz, 1H, biph 4′-H), 7.51-7.55 (m, 2H, biph 3′-H, 5′-H), 7.79 (d, *J* = 7.7 Hz, 2H, biph 2′-H, 6′-H), 7.96 (d, *J* = 8.4 Hz, 2H, biph 2-H, 6-H), 8.17 (d, *J* = 8.3 Hz, 2H, biph 3-H, 5-H), 9.31 (t, *J* = 5.7 Hz, 1H, NH). MS (EI, 140 °C): m/z (%) = 323 (44) [$M^{+\bullet}$], 294 (11), 239 (13), 224 (13), 23 (18), 181 (38), 180 (31), 179 (100), 153 (17), 152 (22) 151 (11), 74 (49), 56 (17), 44 (10), 31 (19).

*N-[2-(2-Hydroxyethylamino)-ethyl]-5-(biphenyl-4-yl)-1,3,4-oxadiazole-2-carboxamide (**6g**)*

From 800 mg (2.7 mmol) **5a** (method C). Crystals, mp. 157 °C (methanol), yield 570 mg (60%). Anal. $C_{19}H_{20}N_4O_3$ (352.4). IR (KBr): ν = 1688 cm^{-1} (C=O); 1611 (C=N). ^{1}H-NMR / 400 MHz ([D$_6$]DMSO): δ (ppm) = 2.61 (t, *J* = 5.8 Hz, 2H, CH_2-CH_2OH), 2.74 (t, *J* = 6.5 Hz, 2H, CH_2-CH_2NHCO), 3.39 (t, *J* = 6.4 Hz, 2H, CH_2-NHCO), 3.45 (br. s, 2H, CH_2-OH), 4.45 (br. s, 1H, OH, D_2O exchange), 7.43−7.47 (m, 1H, biph 4′-H), 7.51−7.55 (m, 2H, biph 3′-H, 5′-H), 7.76−7.80 (m, 2H, biph 2′-H, 6′-H), 7.94−7.97 (m, 2H, biph 2-H, 6-H), 8.16−8.18 (m, 2H, biph 3-H, 5-H), 9.25 (br. s, 1H, NHCO, D_2O exchange). MS (EI, 150 °C): m/z (%) = 352 (<1) [$M^{+\bullet}$], 303 (13), 221 (21), 181 (54), 179 (27), 153 (14), 152 (18), 87 (11), 74 (100) [$C_3H_8NO^+$], 56 (33), 30 (12).

*N-(3-Dimethylaminopropyl)-5-hexyl-1,3,4-oxadiazole-2-carboxamide (**6h**)*

From 810 mg (3.5 mmol) **5b** (method B). Powder, mp. 34 °C (eluent: dichloromethane/methanol 10:1), yield 890 mg (90%). Anal. $C_{14}H_{26}N_4O_2$ (282.4). IR (KBr): ν = 1690 cm^{-1} (C=O); 1562 (C=N). ^{1}H-NMR / 400 MHz ([D$_6$]DMSO): δ (ppm) = 0.86 (t, *J* = 6.7 Hz, 3H, CH_3), 1.28−1.36 (m, 6H, $(CH_2)_3CH_3$), 1.61−1.75 (m, 4H, CH_2-CH_2N u. CH_2-CH_2Ox), 2.13 (s, 6H, $(CH_3)_2$N), 2.25 (t, *J* = 6.7 Hz, 2H, CH_2-N$(CH_3)_2$), 2.91 (t, *J* = 7.5 Hz, 2H, CH_2Ox), 3.25−3.29 (m, after D_2O exchange t, *J* =7.0 Hz, 2H, CH_2-NHCO), 9.26−9.29 (m, 1H, NH, D_2O exchange). MS (EI, 40 °C): m/z (%) = 282 (<1) [$M^{+\bullet}$], 58 (100) [$C_3H_8N^+$].

*N-(3-Dimethylaminopropyl)-5-(3-phenylpropyl)-1,3,4-oxadiazole-2-carboxamide (**6i**)*

From 520 mg (2.0 mmol) **5c** (method C). Crystals, mp. 47.5 °C (methanol), yield 490 mg (78%). Anal. $C_{17}H_{24}N_4O_2$ (316.4). IR (KBr): ν = 1689 cm^{-1} (C=O); 1561 (C=N). ^{1}H-NMR / 400 MHz ([D$_6$]DMSO): δ (ppm) = 1.61−1.66 (m, 2H, CH_2-CH_2N), 2.00-2.07 (m, 2H, CH_2-CH_2Ph), 2.12 (s, 6H, $(CH_3)_2$N), 2.24 (t, *J* = 7.0 Hz, 2H, CH_2-N$(CH_3)_2$), 2.66−2.70 (m, 2H, CH_2-Ph), 2.92 (t, *J* = 7.5 Hz, 2H, CH_2Ox), 3.25−3.28 (m, after D_2O exchange t, *J* =7.0 Hz, 2H, CH_2-NHCO), 7.17−7.31 (m, 5H, Ph), 9.24−9.25 (m, 1H, NH, D_2O exchange). MS (EI, 100 °C): m/z (%) = 316 (2) [$M^{+\bullet}$], 58 (100) [$C_3H_8N^+$].

*N-[2-(1-Methylpyrrolidin-2-yl)-ethyl]-5-(biphenyl-4-yl)-1,3,4-oxadiazole-2-carboxamide (**6j**)*

From 520 mg (1.8 mmol) **5a** (method C). Crystals, mp. 126 °C (methanol), yield 430 mg (65%). Anal. $C_{22}H_{24}N_4O_2$ (376.5). IR (KBr): ν = 1687 cm^{-1} (C=O); 1611 (C=N). ^{1}H-NMR / 400 MHz ([D$_6$]DMSO): δ (ppm) = 1.43-1.53 (m, 2H, CH_2pyrr), 1.60−1.65 (m, 2H, pyrr-CH_2), 1.86−1.97 (m, 2H, pyrr-CH_2), 2.02−2.12 (m, 2H, pyrr-CH_2), 2.22 (s, 3H, CH_3N), 2.92−2.99 (m, 1H, pyrr 2-H), 3.34−3.37 (m, 2H, CH_2-NH), 7.43−7.47 (m, 1H, biph 4′-H), 7.51−7.55 (m, 2H, biph 3′-H, 5′-H), 7.78−7.80 (m, 2H, biph 2′-H, 6′-H), 7.96 (d, *J* = 8.4 Hz, 2H, biph 2-H, 6-H), 8.16 (d, *J* = 8.4 Hz, biph 3-H, 5-H), 9.44 (t, *J* = 5.7 Hz, 1H, NH, D_2O exchange). MS (EI, 160 °C): m/z (%) = 376 (3) [$M^{+\bullet}$], 84 (100) [Pyrr$^+$].

*Synthesis of the carboxamidoximes **8** (procedure from Knudsen [8] modified)*

13.9 g hydroxylamine hydrochloride (0.2 mol) and 10.6 g Na_2CO_3 (0.1 mol) were dissolved in 50 mL of water. The mixture was added to the corresponding nitrile (0.1 mol) in 50 mL ethanol. After stirring for 64 h at a temperature of 30 to 40 °C the mixture was filtered off. The filtrate was concentrated in vacuo at a temperature of less than 35 °C. Then 1 N HCl was added. In order to remove rests of the nitrile the solution was washed with ether or ethylacetate. Now a saturated solution of Na_2CO_3 was added until a pH-value of 7−8 is reached. The mixture was extracted with ether, dried and the solvent removed in vacuo.

*4-Biphenyl-1-carboxamidoxime (**8a**)*

From 17.9 g (0.10 mol) 4-biphenyl-1-carbonitrile, extracted with ethylacetate. Crystals (ethylacetate/petroleum ether), mp. 165 °C, yield 8 g (38%). Anal. $C_{13}H_{12}N_2O$ (212.3). IR (KBr): ν = 1643 cm^{-1} (C=N). ^{1}H-NMR/400 MHz ([D$_6$]DMSO): δ (ppm) = 5.84 (s, 2H, NH_2, D_2O exchange), 7.38 (t, *J* = 7.4 Hz, 1H, Biph 4′-H), 7.45-7.49 (m, 2H, biph 3′-H, 5′-H), 7.67−7.71 (m, 4H, biph 2-H-, 6-H, 2′-H, 6′-H), 7.77 (d, *J* = 8.4 Hz, 2H, biph 3-H, 5-H), 9.67 (s, 1H, OH, D_2O exchange). MS (EI, 40 °C): m/z (%) = 213 (15), 212 (100) [$M^{+\bullet}$], 196 (18), 195 (96) [$M^{+\bullet}$-OH$^\bullet$ respectively NH_3], 180 (37) [$M^{+\bullet}$-$H_2NO^\bullet$], 179 (17), 166 (15), 153 (14), 152 (35), 151 (13), 97 (10), 76 (11).

*1-Naphthaline-carboxamidoxime (**8b**)*

From 15.3 g (0.10 mol) 1-naphthaline-carbonitrile. Crystals (ether), mp. 146 °C ([21] 148-149 °C), yield 5.6 g (30%). Anal. $C_{11}H_{10}N_2O$ (186.1). IR (KBr): ν = 1653 cm^{-1} (C=N). ^{1}H-NMR / 400 MHz ([D$_6$]DMSO): δ (ppm) = 5.96 (s, 2H, NH_2, D_2O exchange), 7.50−8.32 (m, 7H, naphth), 9.55 (s, 1H, OH, D_2O exchange). MS (EI, 35 °C): m/z (%) = 186 (76) [$M^{+\bullet}$], 170 (16), 169 (100) [$M^{+\bullet}$-OH$^\bullet$ respectively NH_3], 168 (17), 155 (13), 154 (58) [$M^{+\bullet}$-$H_2NO^\bullet$], 153 (23), 141 (11), 140 (14), 128 (13), 127 (48), 126 (20), 114 (11), 84 (10), 77 (21), 63 (11), 32 (66).

*Synthesis of O-ethyloxalyl-carboxamidoxime **10** (procedure of Palazzo [9] modified)*

The carboxamidoxime (0.01 mol) was dissolved in anhydrous acetone and mixed with equivalent amounts of K_2CO_3. A solution of oxalic acid ethyl ester chloride (0.01 mol) in 5 mL of acetone was added dropwise while cooling with ice and stirring for 30 min. After stirring for 2 h at room temperature the solvent was removed in vacuo. The product was dissolved in dichloromethane, washed with water and 0.1 N HCl and dried with Na_2SO_4. The solvent was removed in vacuo. The product was recrystallized from ether/petroleum ether.

*O-Ethyloxalyl-biphenyl-4-yl-carboxamidoxime (**10a**)*

From 5 g (23.6 mmol) **8a**. Powder, mp. 112 °C (dichloromethane), yield 4.8 g (65%). Anal. $C_{17}H_{16}N_2O_4$ (312.4). IR (KBr): ν = 1766 cm^{-1} (C=O); 1746 (C=O); 1633 (C=N). ^{1}H-NMR / 400 MHz ([D$_6$]DMSO): δ (ppm) = 1.31 (t, *J* = 7.2 Hz, 3H, CH_3), 4.32 (q, *J* = 7.1 Hz, 2H, CH_2CH_3), 7.13 (br. s, 2H, NH_2, D_2O exchange), 7.41 (t, *J* = 7.3 Hz, 1H, biph 4′-H), 7.48−7.52 (m, 2H, biph 3′-H, 5′-H), 7.72−7.74 (m, 2H, biph 2′-H, 6′-H), 7.77−7.82 (m, 4H, biph 2-H, 6-H, 3-H, 5-H). MS (EI, 100 °C): m/z (%) = 312 (<1) [$M^{+\bullet}$], 295 (20), 294 (100) [$M^{+\bullet}$-H_2O], 195 (16), 181 (18), 154 (10).

*O-Ethyloxalyl-1-naphthyl-carboxamidoxime (**10b**)*

Ether was used for the final extraction. From 5 g (26.9 mmol) **8b**. Crystals, mp. 75 °C, yield 2.4 g (31%). Anal. $C_{15}H_{14}N_2O_4$ (286.3). IR (KBr): ν = 1772 cm^{-1} (C=O); 1743 (C=O); 1627 (C=N). ^{1}H-NMR / 400 MHz ([D$_6$]DMSO): δ (ppm) = 1.30 (t, *J* = 7.1 Hz, 3H, CH_3), 4.30 (q, *J* = 7.1 Hz, 2H, CH_2CH_3), 7.29 (br. s, 2H, NH_2, D_2O exchange), 7.57−8.25 (m, 7H, naphth). MS (EI, 80 °C): m/z (%) = 286 (<1) [$M^{+\bullet}$], 269 (18), 268 (100) [$M^{+\bullet}$-H_2O], 195 (37),

169 (14), 168 (35), 153 (27), 140 (18), 127 (23) [$C_{10}H_7^+$], 126 (13), 31 (12), 29 (45) [$C_2H_5^+$].

Synthesis of the 1,2,4-oxadiazole-5-carboxylic acid ethyl ester **11**

The O-ethyloxalyl-carboxamidoxime was refluxed in toluene for 2 h with separation of the water formed. After having removed the solvent in vacuo there was a final recrystallization in the solvent stated.

*3-(Biphenyl-4-yl)-1,2,4-oxadiazole-5-carboxylic acid ethyl ester (**11a**)*

From 3.5 g (11.2 mmol) **10a**. Powder, mp. 74 °C (ether/petroleum ether), yield 2.1 g (65%). Anal. $C_{17}H_{14}N_2O_3$ (294.3). IR (KBr): ν = 1749 cm^{-1} (C=O); 1611 (C=N). ^{1}H-NMR/400 MHz ([D_6]DMSO): δ (ppm) = 1.39 (t, *J* = 7.1 Hz, 3H, CH_3), 4.48 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 7.43−7.48 (m, 1H, biph 4′-H), 7.51−7.55 (m, 2H, biph 3′-H, 5′-H), 7.78 (d, *J* = 7.8 Hz, 2H, biph 2′-H, 6′-H), 7.92 (d, *J* = 8.3 Hz, 2H, biph 2-H, 6-H), 8.15 (d, *J* = 8.2 Hz, 2H, aromat. 3-H, 5-H). **MS** (EI, 110 °C): m/z (%) = 295 (20), 294 (100) [$M^{+\bullet}$], 195 (12), 179 (12).

*3-(1-Naphthyl)-1,2,4-oxadiazole-5-carboxylic acid ethyl ester (**11b**)*

From 2.4 g (8.4 mmol) **10b**. Slightly violet powder, mp. 45 °C (ether/petroleum ether), yield 2.0 g (89%). Anal. $C_{15}H_{12}N_2O_3$ (268.3). IR (KBr): ν = 1748 cm^{-1} (C=O); 1577 (C=N). ^{1}H-NMR / 400 MHz ([D_6]DMSO): δ (ppm) = 1.40 (t, *J* = 7.1 Hz, 3H, CH_3), 4.50 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 7.66−8.77 (m, 7H, naphth). MS (EI, 45 °C): m/z (%) = 268 (25) [$M^{+\bullet}$], 195 (11), 168 (11), 154 (14), 153 (100) [$C_{11}H_7N^+$], 127 (13), 126 (20), 63 (11), 57 (11).

*General procedure of the synthesis of 1,2,4-oxadiazole-5-carboxamides **12***

The same procedure as described for the synthesis of the 1,3,4-oxadiazole-2-carboxamides **6** was used.

*N-(3-Dimethylaminopropyl)-3-(biphenyl-4-yl)-1,2,4-oxadiazole-5-carboxamide (**12a**)*

From 560 mg (1.9 mmol) **10a** (method C). Powder, mp. 79 °C (dichloromethane), yield 450 mg (68%). Anal. $C_{20}H_{22}N_4O_2$ (350.4). IR (KBr): ν = 1697 cm^{-1} (C=O); 1574 (C=N). ^{1}H-NMR / 400 MHz ([D_6]DMSO): δ (ppm) = 1.67-1.74 (m, 2H, CH_2-CH_2N), 2.18 (s, 6H, $(CH_3)_2$N), 2.32 (t, *J* = 6.9 Hz, 2H, CH_2-N$(CH_3)_2$), 3.36 (after D_2O exchange t, *J* = 7.0 Hz, 2H, CH_2-NH), 7.42-7.46 (m, 1H, biph 4′-H), 7.51-7.54 (m, 2H, biph 3′-H, 5′-H), 7.77-7.79 (m, 2H, biph 2′-H, 6′-H), 7.92-7.94 (m, 2H, biph 2-H, 6-H), 8.14-8.16 (m, 2H, biph 3-H, 5-H), 9.61 (t, *J* =5.7 Hz, 1H, NH, D_2O exchange). MS (EI, 90 °C): m/z (%) = 350 (3) [$M^{+\bullet}$], 212 (14), 195 (12), 179 (10), 58 (100) [$C_3H_8N^+$], 32 (12), 28 (50).

*N-(2-Dimethylaminoethyl)-3-(biphenyl-4-yl)-1,2,4-oxadiazole-5-carboxamide (**12b**)*

From 580 mg (2.0 mmol) **11a** (method A). Shining crystals, mp. 97 °C (methanol), yield 244 mg (37%). Anal. $C_{19}H_{20}N_4O_2$ (336.4). IR (KBr): ν = 1695 cm^{-1} (C=O); 1578 (C=N). ^{1}H-NMR / 400 MHz ($CDCl_3$): δ (ppm) = 2.75 (s, 6H, $(CH_3)_2$N), 3.12 (s, 2H, CH_2-N$(CH_3)_2$), 3.89−3.90 (m, 2H, CH_2-NHCO), 7.38−7.42 (m, 1H, biph 4′-H), 7.45−7.49 (m, 2H, biph 3′-H, 5′-H), 7.63−7.65 (m, 2H, biph 2′-H, 6′-H), 7.71−7.73 (m, 2H, biph 2-H, 6-H), 8.20−8.22 (m, 2H, biph 3-H, 5-H), 8.81 (br. s, 1H, NH, D_2O exchange.). MS (EI, 110 °C): m/z (%) = 336 (1) [$M^{+\bullet}$], 58 (100) [$C_3H_8N^+$].

*N-(3-Diethylaminopropyl)-3-(biphenyl-4-yl)-1,2,4-oxadiazole-5-carboxamide (**12c**)*

From 520 mg (1.8 mmol) **11a** (method C). Oil, yield 270 mg (41%). Anal. $C_{22}H_{26}N_4O_2$ (378.5). IR (film): ν = 1697 cm^{-1} (C=O); 1575 (C=N). ^{1}H-NMR / 400 MHz ([D_6]DMSO): δ (ppm) = 0.98 (t, *J* = 7.1 Hz, 6H, 2 x CH_3), 1.66−1.73 (m, 2H, CH_2-CH_2N), 2.46-2.48 (m, 6H, 3 x CH_2N), 3.38 (after D_2O exchange br. s, 2H, 2H, CH_2-NH), 7.42−7.46 (m, 1H, biph 4′-H), 7.50−7.54 (m, 2H, biph 3′-H, 5′-H), 7.76−7.79 (m, 2H, biph 2′-H, 6′-H), 7.92−7.94 (m, 2H, biph 2-H, 6-H), 8.13−8.15 (m, 2H, biph 3-H, 5-H), 9.71 (s, 1H, NH, D_2O exchange). MS (EI, 90 °C): m/z (%) = 378 (4) [$M^{+\bullet}$], 86 (100) [$C_5H_{12}N^+$].

*N-[3-(2-Hydroxyethylamino)-propyl]-3-(biphenyl-4-yl)-1,2,4-oxadiazole-5-carboxamide (**12d**)*

From 540 mg (1.8 mmol) **11a** (method C). Crystals, mp. 114 °C (ethylacetate), yield 370 mg (56%). Anal. $C_{20}H_{22}N_4O_3$ (366.4). IR (KBr): ν = 1683 cm^{-1} (C=O); 1577 (C=N). ^{1}H-NMR / 400 MHz ([D_6]DMSO): δ (ppm) = 1.66−1.73 (m, 2H, CH_2-CH_2-CH_2), 2.57−2.63 (m, 4H, CH_2-NH-CH_2), 3.39 (after D_2O exchange t, *J* = 6.9 Hz, 2H, CH_2-NHCO), 3.47 ("q", *J* = 5.4 Hz, 2H, CH_2-OH, after D_2O exchange t, *J* = 5.8 Hz), 4.46 (t, *J* = 5.2 Hz, 1H, OH, D_2O exchange), 7.44 (t, *J* = 7.3 Hz, 1H, biph 4′-H), 7.51−7.54 (m, 2H, biph 3′-H, 5′-H), 7.77 (d, *J* = 7.6 Hz, 2H, biph 2′-H, 6′-H), 7.92 (d, *J* = 8.4 Hz, 2H, biph 2-H, 6-H), 8.15 (d, *J* = 8.3 Hz, 2H, biph 3-H, 5-H), 9.50 (br. s, 1H, NHCO, D_2O exchange). MS (EI, 80 °C): m/z (%) = 366 (<1) [$M^{+\bullet}$], 336 (22); 335 (100) [$M^{+\bullet}$-CH_2OH], 222 (24), 195 (13), 180 (15), 179 (71), 178 (13), 153 (10), 152 (13), 140 (12), 113 (40), 76 (10), 74 (32) [$C_3H_8NO^+$], 70 (15), 56 (24), 44 (48) [$C_2H_4O^+$], 43 (13), 42 (10), 41 (10), 30 (18), 28 (16).

*N-(3-Dimethylaminopropyl)-3-(1-naphthyl)-1,2,4-oxadiazole-5-carboxamide (**12e**)*

From 550 mg (2.1 mmol) **11b** (method C). Brown oil, yield 406 mg (61%). Anal. $C_{18}H_{20}N_4O_2$ (324.4). IR (film): ν = 1691 cm^{-1} (C=O); 1574 (C=N). ^{1}H-NMR / 400 MHz ([D_6]DMSO): δ (ppm) = 1.68−1.75 (m, 2H, CH_2-CH_2N), 2.16 (s, 6H, $(CH_3)_2$N), 2.30 (t, *J* = 6.9 Hz, 2H, CH_2-N$(CH_3)_2$), 3.34−3.40 (m, 2H, CH_2-NH), 7.67−8.78 (m, 7H, naphth), 9.60 (br. s, 1H, NH, D_2O exchange). MS (EI, 130 °C): m/z (%) = 324 (4) [$M^{+\bullet}$], 58 (100) [$C_3H_8N^+$].

*N-(3-Methylaminopropyl)-3-(biphenyl-4-yl)-1,2,4-oxadiazole-5-carboxamide hydrochloride (**12f**)*

From 580 mg (2.0 mg) **11a** (method B). Crystals, mp. 209 °C (methanol/few HCl_{konz}), yield 500 mg (68%). Anal. $C_{19}H_{21}ClN_4O_2$ (372.9). IR (KBr): ν = 1683 cm^{-1} (C=O); 1577 (C=N). ^{1}H-NMR/ 400 MHz ([D_6]DMSO): δ (ppm) = 1.88−1.95 (m, 2H, CH_2-CH_2N), 2.54−2.56 (m, 3H, CH_3), 2.96 (br. s, 2H, CH_2-NH_2CH_3), 3.39-3.43 (m, 2H, CH_2-NHCO), 7.43−7.46 (m, 1H, biph 4′-H), 7.51−7.55 (m, 2H, biph 3′-H, 5′-H), 7.77−7.79 (m, 2H, biph 2′-H, 6′-H), 7.93−7.95 (m, 2H, biph 2-H, 6-H), 8.15−8.17 (m, 2H, biph 3-H, 5-H), 8.73 (br. s, 2H, NH_2, D_2O exchange), 9.61 (t, *J* = 5.8 Hz, 1H, NHC=O, D_2O exchange). MS (EI, 140 °C): m/z (%) = 336 (<1) [$M^{+\bullet}$ der Base], 180 (10), 179 (42), 152 (13), 85 (19), 70 (16), 57 (12), 44 (100) [$C_2H_6N^+$], 43 (11), 28 (28).

Biology

Born test

Preparation of the blood plasmas

Freshly drawn venous human citrated blood (1 pt sodium citrate solution 3, 13%, Fa. Eifelfango, Neuenahr, 9 pts blood) from

healthy subjects who had not taken acetylsalicylic acid or other drugs with antiplatelet activity for 10 days was centrifuged (Micro 20, A. Hettich GmbH, Tutlingen) with 100 *g* (800 rpm) for platelet rich plasma (PRP) or 2000 *g* (Biofuge A, Haereus, 12 500 rpm) for platelet poor plasma (PPP).

Platelet aggregation procedures [12, 13]

Platelet aggregation induced by collagen

At first the concentration of collagen fibrils which induce maximum aggregation of the platelets is determined. To 200 µL of PRP 20 µL of hepes buffer i.e. 2-[4-(2-Hydroxyethyl)-piperazin-1-yl]-ethane-sulfonic acid 0.001 M (= 238.3 mg/L) Fa. Sigma (without test compound) are added and the mixture incubated 4 min at 37.4 °C. Now the cuvette is put in the channel of the APACT aggregometer (Automated Platelet Aggregation and Coagulation Tracer, Biochemica GmbH, D-65558 Flacht) with software APACT professional version 1.1.

While automatically stirred by a small magnet 20 µL of the aggregation inducer collagen Horm®, (Nycomed Pharma GmbH), in hepes buffer which contain 0.25 µg fibrils are added. The solution is obtained by dilution of the stock solution containing 1 mg fibrils/mL with hepes. Now the change in light transmission is recorded and generally the maximum aggregation response observed. To assure this, the procedure is repeated with 0.32 µg fibrils/20 µL. On the other hand the procedure is repeated with 0.125 µg fibrils/20 µL or 0.16 µg fibrils/20 µL. To check the correct function of the test system the influence of a standard aggregation inhibitor namely DL-lysine monoacetyl salicylate (Bayer) on the platelet aggregation is determined as if it were a test compound.

The test compound (or the standard inhibitor) is dissolved in hepes buffer. Then 20 µL of the test solution is given to 200 µL PRP in the test cuvette and incubated 4 min at 37.4 °C. Then 20 µL of the inducer in the concentration determined above is added and the change in light transmission recorded. The percentage of aggregation is determined as the ratio of heights of the aggregation curves with and without the test compound. Each curve is corrected automatically for the light absorption of platelet poor plasma (PPP) of the same donor. If the test compound is not totally soluble in hepes buffer DMSO is added. It is carefully assured that the final concentration of DMSO in the test cuvette is below 0.3% as in higher concentrations DMSO itself is a platelet aggregation inhibitor. By dilution of the stem solutions of the test compound in 1:1 steps with hepes buffer its concentration is bisected in each step and measured again so that the percentage of aggregation as function of the concentration of the test compound is obtained. These values are plotted in a semilogarithmic scale [% = f (lg c)] and the corresponding aggregation curves obtained. When a line at the 50% value is drawn parallel to the x-axis an intersection with the aggregation curve is got. At this point a perpendicular is raised upon the x-axis and the IC_{50} value directly read off from the x-axis. The standard deviation is determined from the standard inhibitors with n = 10 and is found generally to be <10% rel. The assay with the test compounds was mostly run only in duplicate provided that the difference of the values obtained was below 10%. The IC_{50} value for the asa lysinate is 175 ± 20 µM.

Platelet aggregation with other inducers

The final concentration of the inducer in the test cuvette and the IC_{50} values of the standard inhibitors are (µM): ADP 0.5−1.0/NECA 1.0; adrenaline 0.1−1.0/phentolaminemesylate 2.0; PAF 0.25−1.0/apafant (WEB 2086) 0.6; serotonin 0.1−2.0 / imipramine 2.0.

Acknowledgments

We are indebted to Mrs. Nora Reitner and Mrs. Heike Scheffler for the skilful performance of the biological tests. We thank Dr. H.-P. Stasch and Dr. E. Bischoff for the s-GC and PDE tests.

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