Full Paper

New 1*H*-Pyrazole-4-Carboxamides with Antiplatelet Activity

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Nine title compounds were synthesized and investigated in the Born test for their antiplatelet activities against collagen, ADP, adrenaline, and platelet activating factor (PAF) as inducers of the aggregation. Using collagen three compounds with IC_{50} values below 100 μM were found (**3b**, **3e**, **3i**). Activities in nanomolar concentrations were observed against ADP (**3b**, IC_{50} = 9.4 nM), adrenaline (**3i**, IC_{50} = 5.8 nM), and platelet activating factor (**3e**, IC_{50} = 0.45 nM).

Keywords: ADP / Adrenaline / Antiplatelet properties / 1*H*-Pyrazole-4-carboxamides / PAF antagonism

Received: March 14, 2008; accepted: September 29, 2008

DOI 10.1002/ardp.200800181

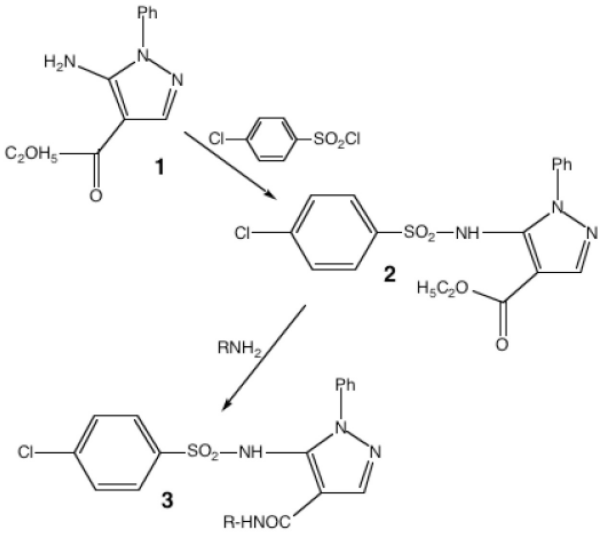
Introduction

In a number of previous publications, we were able to show that the substitution of heterocycles rich in nitrogen like purines [1], indazoles [2], triazoles [3], oxadiazoles [4], imidazoles [5], pyrimidocinnolines [6], phthalazines [7], or thiazoles [8] with a carboxamide partial structure in addition to basic groups leads to a wide variety of compounds with antiplatelet activities in micromolar concentrations. In this paper, we wish to report a number of pyrazole derivatives fulfilling these structural requirements and, consequently, were promising to show remarkable antiplatelet activities.

Results and discussion

Chemistry

The synthesis of the title pyrazole-4-carboxamides is shown in Scheme 1. Starting material is the commercially available ethyl 5-amino-1-phenyl-1*H*-4-pyrazolecarboxylate **1**. This compound is converted with 4-chlorophenylsulfonic acid chloride to the sulfonamide **2**. Aminolysis with suitable amines R-NH_2 yielded the type **3** title compounds.



Scheme 1. Synthesis of type **3** pyrazole-4-carboxamides.

Biology

The antiplatelet effects obtained in the Born test with compounds **3a**–**3i** are summarized in Table 1. As inducers of the platelet aggregation, we used collagen, adenosindiphosphate (ADP), adrenaline, or platelet activating factor (PAF), respectively. The rationale for design-

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Abbreviations: NECA = 5-(*N*-ethylcarboxamido) adenosine; platelet activating factor (PAF); platelet-rich plasma (PRP); platelet-poor plasma (PPP)

Table 1. Inhibition of platelet aggregation induced by collagen, adrenaline, ADP, or PAF by selected type **3** pyrazole-4-carboxamides.

Compound	R	Collagen	**IC_{50} (μM) ADP**	Adrenaline	PAF
3a	H_3C-NH-$(CH_2)_2$-	110	**0.5**	10	> 300
3b	H_5C_2-NH-$(CH_2)_2$-	> 300	**0.094**	**0.58**	240
3c	H_9C_4-NH-$(CH_2)_2$-	95	215	1.25	7.5
3d	$H_{13}C_6$-NH-$(CH_2)_2$-	> 300	30	30	55
3e	Cyclohexyl-NH-$(CH_2)_3$-	28	94	14	**0.00045**
3f	Ph-NH-$(CH_2)_2$-	120	45	75	56
3g	Ph-CH_2-piperidin-4-yl	125	65	**0.12**	56
3h	H_3CO-$(CH_2)_2$-	105	125	1.05	32
3i	H_3CO-$(CH_2)_3$-	50	**0.54**	**0.0058**	12.5
Asa	–	175	–	–	–
NECA[§]	–	–	1	–	–
Phentolamine	–	–	–	2	–
Apafant (WEB-2086)	–	–	–	–	0.6

Incubation time 20′, Standard deviation ≤ 10%; § NECA = 5-(*N*-Ethylcarboxamido)-adenosine.

Table 1a. Parameters for the rule-of-five for pyrazoles **3a**–**3i**.

Compound	Molecular weight (g/mol)	clogP	Number of H-bond acceptors (N/O)	Number of H-bond donors (NH/OH)	Chance of good oral biovailability proposed
3a	433.9	2.14	8	3	yes
3b	447.9	2.16	8	3	yes
3c	476.0	3.22	8	3	yes
3d	**504.1**	4.23	8	3	yes
3e	**516.1**	4.31	8	3	yes
3f	496.0	3.83	8	3	yes
3g	**536.1**	4.47	8	2	yes
3h	434.9	2.33	8	2	yes
3i	448.9	2.61	8	2	yes

Lipohilicity was calculated using the software "molinspiration property calculator" (Molinspiration Cheminformatics, Slovensky Grob, Slovak Republic). Violations of the rule-of-five are illustrated in bold style.

ing compounds **3a**–**3e** was to check the influence of R = (cyclo)alkylaminoalkyl. In **3f** and **3g**, the presence of an aromatic moiety in R was investigated. Compounds **3h** and **3i** were synthesized to test the suitablility of a methylether function with different spacers instead of alkylamino groups for antiplatelet activities.

In general, we observe only small effects against collagen. It is, however, interesting that **3e** with a cyclohexylaminopropyl function which was here selected from the thiazole derivatives [8] is as well the most active compound with respect to collagen.

A very thrilling pattern of effects was observed with inducers other than collagen. When ADP is used, compound **3b** shows an IC_{50} = 94 nM. Compounds **3a** and **3i** still show an activity <1 μM. The other compounds show smaller effects between IC_{50} = 30–215 μM. As **3b** additionally has an IC_{50} = 0.58 μM and **3i** an IC_{50} = 5.8 nM against adrenaline both compounds can be defined as mixed ADP / adrenaline antagonists. Compound **3g** appears as specific adrenaline antagonist. In contrast, **3e** (IC_{50} = 0.45 nM) is a potent and specific PAF antagonist.

Since this is our last paper in the series of antiplatelet agents, a comprehensive view on structure-activity relationships appears indicated. As this has already been done for collagen as inducer of the platelet aggregation [8], the effects against ADP, adrenaline, and PAF (platelet activating factor) will be discussed. Therefore, Figure 1 summarizes the most active compounds for each heterocyclic system identified in our previous investigations including the characteristic positions of the substituents. The standard inhibitors are NECA = 5-(*N*-ethylcarboxamido) adenosine, IC_{50} = 1 μM against ADP, phentolamine (IC_{50} = 2 μM against adrenaline), or apafant (WEB 2086) IC_{50} = 0.6 μM against PAF.

The important structural features are an aromatic heterocycle with several nitrogen and sulfur atoms. This heterocycle has two or three substituents with the following properties: (i) A basic moiety especially –NH-$(CH_2)_3$-NH-

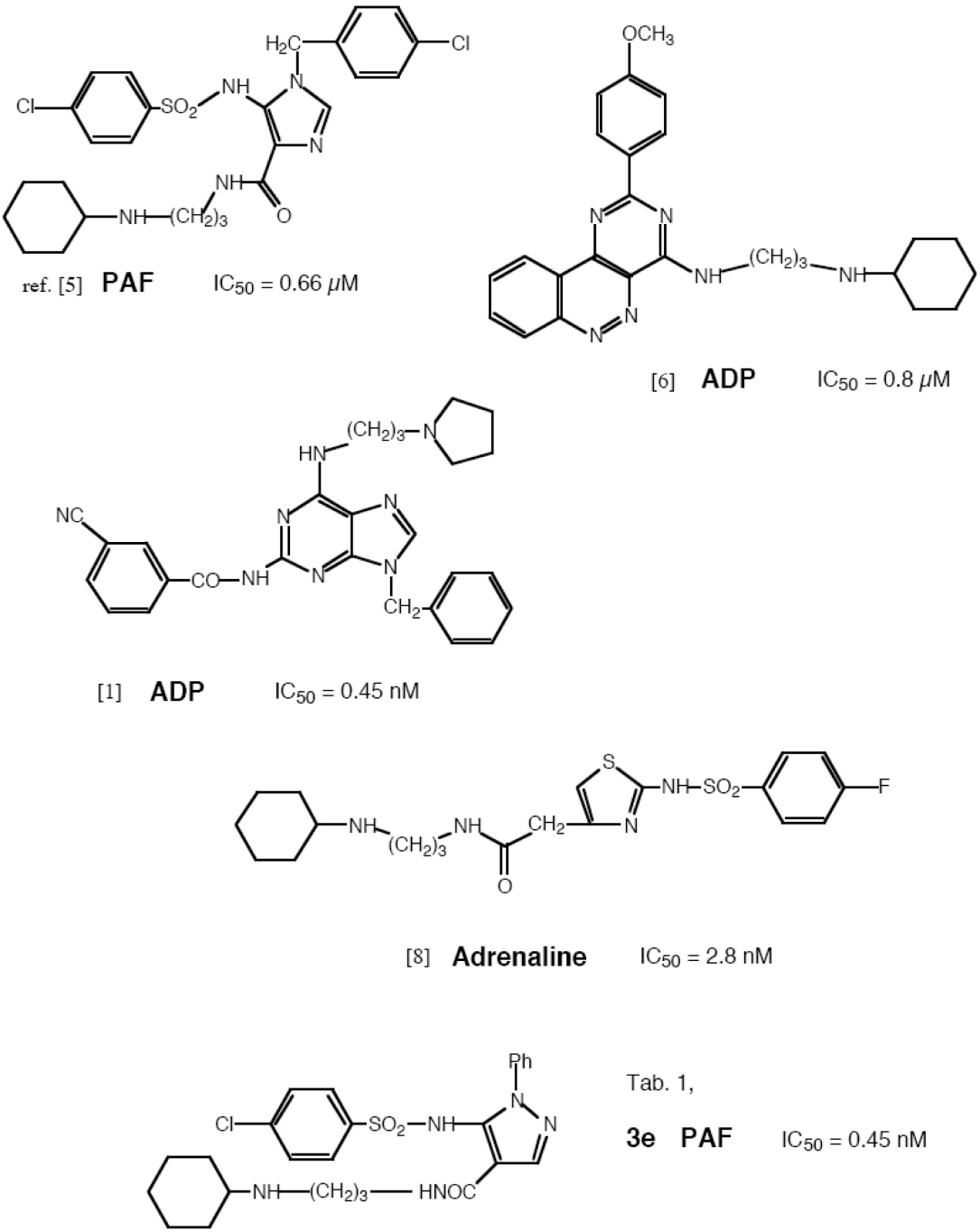

Figure 1. Structure of the most active compounds in each class of heterocycles.

cyclohexyl or 1-pyrrolidinylpropyl-amino either bound directly or via a carboxamido group to the heterocycle; (ii) an aromatic moiety bound via a sulfonamido or carboxamido group or connected directly to the heterocycle. This shows that these groups are suitable spacers but not essential for the antiplatelet activity. (iii) A hydrophobic group, i. e. a phenyl or benzyl rest.

The first two rests are essential while the third one is an additional option (see Figure 1, purines, pyrazole, imidazole).

Using this rationale, a large number of antiplatelet agents were obtained. For each inducer of the platelet aggregation (ADP, adrenaline, PAF) the four most powerful inhibitors so far found are listed in Tables 2/2a, 3/3a,

Table 2. Inhibition of platelet aggregation induced by ADP.

Rank	Ref.	Hetero-cyclus	Amide moiety	Aromatic moiety	Basic moiety	IC_{50} (μM)
1	[1]	purine	CONH	3-cyano-phenyl	1-pyrrolidinyl-$(CH_2)_3$-	0.00045
2	[8]	thiazole	CONH, SO_2NH	4-chloro-phenyl	cyclohexyl-NH-$(CH_2)_2$-	0.0022
3	[1]	purine	CONH	4-(R-NH-SO_2)phenyl	1-pyrrolidinyl-$(CH_2)_2$-	0.0035
4	[8]	thiazole	CONH, SO_2NH	4-chloro-phenyl	cyclohexyl-NH-$(CH_2)_4$-	0.0053

Standard inhibitor NECA = 5-(*N*-ethylcarboxamido)-adenosine, IC_{50} = 1 μM.

Table 2a. Parameters for the Rule-of-Five.

Compound Rank	Molecular weight (g/mol)	clogP	Number of H-bond acceptors (N/O)	Number of H-bond donors (NH/OH)	Chance of good oral biovailability
1	480.6	4.34	9	2	yes
2	457.0	3.65	7	3	yes
3	**592.7**	3.66	**12**	3	**no**
4	485.1	4.12	7	3	yes

Inhibition of platelet aggregation induced by ADP.

Table 3. Inhibition of platelet aggregation induced by adrenaline.

Rank	Ref.	Hetero-cyclus	Amide moiety	Aromatic moiety	Basic moiety	IC_{50} (μM)
1	[1]	purine	CONH	3-cyano-phenyl	cyclohexyl-NH-$(CH_2)_3$-	0.00018
2	[8]	thiazole	CONH, SO_2NH	4-fluoro-phenyl	cyclohexyl-NH-$(CH_2)_3$-	0.0027
3	[8]	thiazole	CONH, CONH	4-fluoro-phenyl	cyclohexyl-NH-$(CH_2)_3$-	0.0028
4	Tab. 1	pyrazole	CONH, SO_2NH	4-chloro-phenyl	H_3CO-$(CH_2)_3$-	0.0058

Standard inhibitor phentolamine, IC_{50} = 2 μM.

Table 3a. Parameters for the Rule-of-Five.

Compound Rank	Molecular weight (g/mol)	clogP	Number of H-bond acceptors (N/O)	Number of H-bond donors (NH/OH)	Chance of good oral biovailability
1	**508.6**	**5.60**	9	3	**no**
2	454.6	3.41	7	3	yes
3	418.5	3.53	6	3	yes
4	448.9	2.61	8	2	yes

Inhibition of platelet aggregation induced by adrenaline.

4/4a. Their chemical structure is given in columns to allow better comparison. Table 2 shows that only two heterocycles, i. e. purine and thiazole, are the structural basis for peak activities in low nanomolar concentration ranges. The basic moieties are similar to each other. The aromatic moieties are substituted with electron withdrawing residues. The binding to the central heterocycle is performed by amide bonds or directly. Table 3 offers the adrenaline inhibitors. In addition to Table 2, now highly active pyrazole derivatives are found. Surprisingly, the methoxypropyl derivative **3i** is active in spite of lacking a basic moiety.

In Table 4 finally, a majority of purine derivatives is concentrated. The only new heterocycle is a pyrazole derivative which inhibits PAF with an IC_{50} = 0.45 nM.

During the evaluation of this paper it has been suggested to determine the suitability for anticoagulant purposes where a good absorption after oral administration is obligatory. This should be performed by the calculation of the lipophilicity (clogP) and extended to the examination of the Rule-of-Five developed by Lipinski *et al.* [11].

It was recognized in Table 1a that, in the pyrazole series dealt with in this paper, all compounds (**3a**–**3i**) are

Table 4. Inhibition of platelet aggregation induced by PAF.

Rank	Ref.	Hetero-cyclus	Amide moiety	Aromatic moiety	Basic moiety	IC_{50} (μM)
1	Tab. 1	pyrazole	SO_2NH, CONH	4-chloro-phenyl	cyclohexyl-NH-$(CH_2)_3$-	0.00045
2	[1]	purine	CONH	3-cyano-phenyl	cyclohexyl-NH-$(CH_2)_3$-	0.001
3	[1]	purine	CONH	3-cyano-phenyl	1-pyrrolidinyl-$(CH_2)_3$-	0.035
4	[1]	purine	CONH	2-furyl	1-pyrrolidinyl-$(CH_2)_3$-	0.074

Standard inhibitor Apafant (WEB 2086) IC_{50} = 0.6 μM.

Table 4a. Parameters for the Rule-of-Five.

Compound Rank	Molecular weight (g/mol)	clogP	Number of H-bond acceptors (N/O)	Number of H-bond donors (NH/OH)	Chance of good oral biovailability
1	**516.1**	4.31	8	3	yes
2	**508.6**	**5.60**	9	3	**no**
3	480.6	4.34	9	2	yes
4	445.5	3.87	9	2	yes

Inhibition of platelet aggregation induced by PAF.

promising for a good oral bioavailability. Exeptions are only previewed for compound **3** (Table 2a), compound **1** (Table 3a), and compound **2** (Table 2a) where 2 of 4 criteria are violated, respectively. The remaining nine most active compounds in Tables 2a, 3a, and 4a fulfill the criteria for good absorption.

The correspondence to the Rule-of-Five may be an important advantage of the newly discovered 1*H*-pyrazole-4-carboxamides. Altogether, the development of this new class of anticoagulant compounds with the possibility of good oral bioavailabilities is promising.

The authors have declared no conflict of interest.

Experimental

Chemistry

M.p. (uncorr.), Linström apparatus (Bühler, Tübingen, Germany), Elemental analysis: Elementar vario EL (Elementaranalysen Systeme, Hanau, Germany), NMR: Bruker DPX 400 (Bruker Bioscience, Billerica, MA, USA), EI-MS: CH-7A-Varian MAT (70 eV; Varian Inc., Palo Alto, CA, USA), FAB-MS: CH-5-DF-MAT-Varian (cf. references 1-8 for further details).

*5-(4-Chlorophenylsulfonylamino)-1-phenyl-1H-pyrazole-4-carboxylicacidethylester **2***

20 mmol of **1** are dissolved in 50 mL freshly destillated pyridine. While stirring 20 mmol (9.1 g) 4-chlorobenzene-sulfonic acid-chloride are added in portions. The mixture is heated to 100°C, kept at this temperature for 72 h and then pored on cold water. A pH = 1-2 is obtained with 20% hydrochloric acid. The precipitate is sucked off, washed with water, and recrystallized from ethanol. Brown crystals, m.p.: 191°C, yield: 11.2 g (70%). Anal. calcd. for $C_{18}H_{16}ClN_3O_4S$ (405.1): C, 53.3; H, 3.9; N, 10.4. Found: C, 53.2; H, 4.2; N, 10.6. ^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.19 (t, *J* = 7.0 Hz, 3H, OCH_2CH_3), 4.01 (q, *J* = 7.1 Hz, 2H, OCH_2), 7.40 (m, 9H, arom.), 7.89 (s, 1H, pyrazole-3H). IR (KBr): λ (cm^{-1}) = 3427; 3063; 2905; 2565; 2260; 1905; 1717 (CO); 1651; 1499; 1392; 1235; 1118; 976; 756; 693; 604. MS (130°C): m/z (%) = 405 (17) [$M^{+\bullet}$], 359 (34), 230 (13), 184 (100), 175 (41), 111 (38), 77 (68).

General procedure for the synthesis of type-3 carboxamides

2.0 g **2** are suspended in 10 – 15 mL of the desired amine and heated at 80 – 100°C with stirring for a time between three days and three weeks. The progress of the reaction is controlled by TLC on SIL G/UV254 plates (Augram[1], Machery-Nagel, Germany) with CH_2Cl_2 / ethylacetate / methanol saturated with NH_3: 5 / 3 / 2 as eluent. After cooling to room temperature the product is dissolved in CH_2Cl_2 and extracted with water. The organic layer is purified by column chromatography on silica gel (63 – 200 μm, Merck, Germany). If the product remains in the aqueous layer, it is precipitated either at pH = 7 or at pH = 1 – 2. If no precipitate forms, the aqueous layer is extracted with ethyl acetate, dried with Na_2SO_4, and concentrated *in vacuo*. After keeping it for some days in the refrigerator at 5°C, the crystals formed are sucked off and washed with small amounts of ethyl acetate. If necessary, the product is recrystallized from the solvent stated.

*5-(4-Chlorophenylsulfonylamino)-N-(2-methylamino-ethyl)-1-phenyl-1H-pyrazole-4-carboxamide **3a***

From 4.0 g (10 mmol) **2**, brown crystals (dichloromethane), m.p.: 202°C, yield: 2.8 g (65%). – Anal. calcd. for $C_{19}H_{20}ClN_5O_3S$ (433.7): C, 52.6; H, 4.6; N, 16.1. Found C, 52.5; H, 4.5; N, 16.3. ^{1}H-NMR (DMSO-d_6): δ (ppm) = 2.59 (s, 3H, H_3CNH), 3.02 (t, *J* = 5.7 Hz, 2H, $CH_3NHCH_2CH_2$), 3.39 (dt, *J* = 5.8/5.8 Hz, 2H, $CONHCH_2CH_2$), 7.12 (m, 1H, 4-ph-H), 7.14 ("d", *J* = 8.4 Hz, 2H, 3,5 ph-H), 7.18 ("d", *J* = 7.8 Hz, 2H, 3,5-suph-H), 7.23 ("d", *J* = 8.5 Hz, 2H, 2,6-ph-H), 7.44 ("d", *J* = 7.5 Hz, 2H, 2,6-suph-H), 7.68 (s, 1H, pyrazole-3H), 8.30 (t, *J* = 5,8 Hz, 1H, $CONHCH_2$). IR (KBr): λ (cm^{-1}) = 3434; 3013; 2746; 1953; 1834; 1650 (CO); 1542; 1458; 1370; 1232; 1127; 1014; 916;

816; 756; 705; 654. MS (230°C): m/z (%) = 433 (2) [M^+], 186 (71), 57 (63), 44 (100), 30 (13).

5-(4-Chlorophenylsulfonylamino)-N-(2-ethylaminoethyl)-1-phenyl-1H-pyrazole-4-carboxamide ***3b***

From 4.0 g (10 mmol) **2**, brown crystals (dichloromethane), m.p.: 217°C, yield: 2.7 g (60%). Anal. calcd. for $C_{20}H_{22}ClN_5O_3S$ (447.5): C, 53.7; H, 4.9; N, 15.6. Found: C, 53.5; H, 4.9; N, 15.5. ^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.17 (t, *J* = 7.2 Hz, 3H, H_3CCH_2), 2.97 (q, *J* = 7.2 Hz, 2H, CH_2CH_3), 3.03 (t, *J* = 5.8 Hz, 2H, $CH_2NHCH_2CH_2$), 3.41 (dt, *J* = 5.8/5.8 Hz, 2H, $CONHCH_2CH_2$), 7.13 (m, 7H, 3,5 ph-H, 4-ph-H, 3,5-suph-H, 2,6-ph-H), 7.43 ("d", *J* = 7.5 Hz, 2H, 2,6-suph-H), 7.68 (s, 1H, pyrazole-3H), 8.23 (t, *J* = 5.8 Hz, 1H, partly D_2O exchange, $CONHCH_2$), 8.62 (brs, 2H, D_2O exchange, $CH_2N^+H_2CH_2$). IR (KBr): ν (cm^{-1}) = 3092; 2851; 2523; 2359; 2057; 1906; 1647 (CO); 1495; 1389; 1255; 1129; 1013; 916; 820; 693; 635. MS (210°C): m/z (%) = 447 (2) [M^+], 186 (60), 71 (75), 58 (100), 30 (21).

N-(2-butylaminoethyl)-5-(4-chlorophenylsulfonylamino)-1-phenyl-1H-pyrazole-4-carboxamide ***3c***

From 5.2 g (13 mmol) **2**, crystals (dichloromethane), m.p.: 229°C, yield: 3.0 g (50%). Anal. calcd. for $C_{22}H_{26}ClN_5O_3S$ (475.8): C, 55.5; H, 5.5; N, 14.7. Found: C, 55.5; H, 5.4; N, 14.7. ^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.87 (t, *J* = 7.2 Hz, 3H,CH_3CH_2), 1.31 (tq, *J* = 7.2/7.2 Hz, 2H, $CH_3CH_2CH_2$), 1.55 (m, *J* = 7.8 Hz, 2H, $CH_3CH_2CH_2$), 2.92 (t, *J* = 7.6 Hz, 2H, $CONHCH_2$), 3.03 (t, *J* = 5.7 Hz, 2H, $CH_3CH_2CH_2CH_2NHCH_2$), 3.42 (dt, *J* = 5.8 Hz, 2H, $CONHCH_2CH_2$), 7.12 (t, 1H, 4-ph-H), 7.14 ("d", *J* = 8.5 Hz, 2H, 3,5-ph-H), 7.18 (m, 6H, arom. 3,5-suph-H, 2,6-ph-H, 2,6-suph-H), 7.69 (s, 1H, pyrazole-3H), 8.25 (t, *J* = 5.8 Hz, 1H, $CONHCH_2$). IR (KBr): ν (cm^{-1}) = 3059; 2934; 2783; 1956; 1637 (CO); 1526; 1451; 1259; 1087; 972; 822; 695; 614. MS (40°C): m/z (%) = 475 (2) [$M^{+\bullet}$], 359 (10), 186 (42), 86 (100), 30 (75).

5-(4-Chlorophenylsulfonylamino)-N-(2-hexylaminoethyl)-1-phenyl-1H-pyrazole-4-carboxamide monohydrate ***3d***

From 5.5 g (14 mmol) **2**, crystals (dichloromethane), m.p.: 188°C, yield: 0.3 g (5%). Anal. calcd. for $C_{24}H_{39}ClN_5O_4S$ (521.8): C, 55.2; H, 6.2; N, 13.4. Found: C, 55.1; H, 5.9; N, 13.5. ^{1}H-NMR (DMSO-d_6): δ (ppm) = 0.83 (t, *J* = 6.8 Hz, 3H, CH_3CH_2), 1.27 (m, 6H, $CH_2CH_2CH_2CH_2CH_2CH_3$), 1.56 (m, *J* = 7.3 Hz, 2H, $CH_2NHCH_2CH_2CH_2CH_2CH_2CH_3$), 2.93 (t, *J* = 7.7 Hz, 2H, $CH_2NHCH_2(CH_2)_4CH_3$), 3.03 (t, *J* = 5.7 Hz, 2H, $CONHCH_2CH_2$), 3.42 (dt, *J* = 5.7/5.7 Hz, 2H, $CONHCH_2CH_2$), 7.13 (m, 7H, arom. 3,5-ph-H, 3,5-suph-H, 4-ph-H, 2,6-ph-H), 7.43 ("d", *J* = 7.4 Hz, 2H, 2,6-suph-H), 7.69 (s, 1H, pyrazole-3H), 8.24 (t, *J* = 5.9 Hz, 1H, partly D_2O exchange, $CONHCH_2$), 8.32 (brs, 2H, D_2O exchange, $CH_2N^+H_2CH_2$). IR (KBr): ν (cm^{-1}) = 3032; 2857; 1943; 1626 (CO); 1496; 1376; 1170; 1014; 908; 783; 674. MS (40°C): m/z (%) = 503 (1) [$M^{+\bullet}$], 333 (11), 186 (24), 158 (58), 99 (100), 30 (41).

5-(4-Chlorophenylsulfonylamino)-N-(3-cyclohexylaminopropyl)-1-phenyl-1H-pyrazole-4-carboxamide ***3e***

From 4.9 g (12 mmol) **2**, crystals by column chromatography, m.p.: 145°C, yield: 0.2 g (3%). Anal. calcd. for $C_{25}H_{30}ClN_5O_3S$ (575.8): C, 52.3; H, 5.3; N, 12.3. Found: C, 52.7; H, 5.3; N, 12.3. ^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.11 (m, 1H, 4a-cyhex-H), 1.26 (m, 4H, 2a, 3a, 5a, 6a-cyhex-H), 1.59 (m, 1H, 4e-cyhex-H), 1.78 (m, 4H, 2e, 3e, 5e, 6e-cyhex-H), 2.04 (m, 2H, $CH_2CH_2CH_2$), 2.95 (brs, 3H, 1-cyhex-H, $NHCH_2$), 3.13 (dt, *J* = 6.2/6.2 Hz, 2H, $CONHCH_2CH_2$), 7.40 (m, 9H arom.), 8.04 (s, 1H, pyrazole-3H), 8.30 (t, *J* = 5.7 Hz, D_2O exchange, $CONHCH_2$), 10.89 (brs, 1H, D_2O exchange, SO_2NH). IR (KBr): ν (cm^{-1}) = 3410; 2939; 2434; 1635 (CO); 1499; 1393; 1257; 1032; 943; 756; 620. MS (35°C): m/z (%) = 515 (5) [$M^{+\bullet}$], 186 (23), 158 (100), 112 (18).

5-(4-Chlorophenylsulfonylamino)-N-(2-phenylaminoethyl)-1-phenyl-1H-pyrazole-4-carboxamide ***3f***

From 4 g (10 mmol) **2**, light brown crystals (ethanol), m.p.: 219°C, yield: 3.0 g (60%). Anal. calcd. for $C_{24}H_{22}ClN_5O_3S$ (495.1): C, 58.1; H, 4.4; N, 14.1. Found: C, 58.0; H, 4.5; N, 14.3. ^{1}H-NMR (DMSO-d_6): δ (ppm) = 3.19 (m, 4H, $-CH_2CH_2NHCO$), 6.79 (m, 3H, arom.), 7.2 (m, 2H arom.), 7.41 (m, 9H arom.), 8.04 (s, 1H, pyrazole-3H), 8.14 (t, *J* = 5,7 Hz, 1H, D_2O exchange, $CONHCH_2$). IR (KBr): ν (cm^{-1}) = 3313; 2928; 2602; 1647 (CO); 1499; 1391; 1254; 1032; 894; 693. MS (220°C): m/z (%) = 495 (10) [$M^{+\bullet}$], 186 (52), 119 (100).

5-(4-Chlorophenylsulfonylamino)-1-phenyl-N-(1-phenylmethyl-4-piperidinyl)-1H-pyrazole-4-carboxamide dihydrate ***3g***

From 5.0 g (12.5 mmol) **2**, light yellow crystals (ethanol), m.p.: 117°C, yield: 1.7 g (25%). Anal. calcd. for $C_{28}H_{32}ClN_5O_5S$ (585.2): C, 57.4; H, 5.5; N, 12.0. Found: C, 57.6; H, 5.2; N, 11.7. ^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.33 (m, 2H, piperidine-3-H), 1.46 (m, 2H, piperidine-5-H), 1.72 (m, 2H, piperidine-2-H), 1.83 (m, 2H, piperidine-6-H), 2.05 (m, 1H, piperidine-4-H), 2.81 (m, 2H, CH_2-piperidine), 7.24 (m, 14H, arom.), 7.64 (s, 1H, pyrazole-3H), 8.26 (d, *J* = 7.3 Hz, 1H, CON*H*-pip). IR (KBr): ν (cm^{-1}) = 3287 cm^{-1}; 2943; 2677; 2358; 1954; 1608 (CO); 1474; 1344; 1129; 1014; 911; 781; 674; 625. MS (250°C): m/z (%) = 549 (5) [$M^{+\bullet}$], 374 (26), 173 (31), 90 (100), 82 (46).

5-(4-Chlorophenylsulfonylamino)-N-(2-methoxyethyl)-1-phenyl-1H-pyrazole-4-carboxamide semihydrate ***3h***

From 4.0 g (10 mmol) **2**, brown crystals, m.p.: 177°C, yield: 2.7 g (60%). Anal. calcd. for $C_{19}H_{20}ClN_4O_{4.5}S$ (443.7): C, 51.4; H, 4.5; N, 12.6. Found: C, 51.3; H, 4.3; N, 12.3. ^{1}H-NMR (DMSO-d_6): δ (ppm) = 3.13 (t, *J* = 5.7 Hz, 2H, $CONHCH_2CH_2$), 3.27 (s, 3H, OCH_3), 3.29 (dt, *J* = 5.9/5.9 Hz, 2H, $CONHCH_2CH_2$), 7.40 (m, 9H, arom.), 7.93 (t, *J* = 5.5 Hz, 1H, D_2O exchange, $CONHCH_2$), 8.02 (s, 1H, pyrazole-3H). IR (KBr): ν (cm^{-1}) = 3379; 2932; 2760; 1952; 1636 (CO); 1500; 1390; 1260; 1120; 968; 829; 662. MS (150°C): m/z (%) = 433 (14) [$M^{+\bullet}$], 360 (49), 229 (16), 184 (100), 158 (100), 77 (30).

5-(4-Chlorophenylsulfonylamino)-N-(3-methoxypropyl)-1-phenyl-1H-pyrazole-4-carboxamide ***3i***

From 4.8 g (12 mmol) **2**, light brown crystals, m.p.: 150°C, yield: 3.4 g (65%). Anal. calcd. for $C_{20}H_{21}ClN_4O_4S$ (448.1): C, 53.5; H, 4.7; N, 12.5. Found: C, 53.2; H, 4.9; N, 12.3. ^{1}H-NMR (DMSO-d_6): δ (ppm) = 1.59 (quint, *J* = 6.6 Hz, 2H, $CH_2CH_2CH_2NHCO$), 3.02 (dt, *J* = 6.5/6.5 Hz, 2H $CONHCH_2$), 3.24 (s, 3H, OCH_3), 3.35 (t, *J* = 6.2 Hz, 2H, OCH_2), 7.39 (m, 9H, arom.), 7.86 (t, *J* = 5.5 Hz, 1H, D_2O exchange, $CONHCH_2$), 7.99 (s, 1H, pyrazole-3H). IR (KBr): ν (cm^{-1}) = 3019; 2930; 2754; 1839; 1626 (CO); 1499; 1388; 1248; 1092; 895; 693. MS (140°C): m/z (%) = 448 (6) [M^+], 359 (26), 230 (17), 184 (100), 158 (100), 77 (27).

Biological assays

Born test

Preparation of the blood plasma

Freshly drawn venous human citrated blood (1 pt sodium citrate solution 3, 13%, Fa. Eifelfango, Neuenahr, Germany, 9 pts blood) from healthy subjects, who had not taken acetylsalicylic acid or other drugs with antiplatelet activity for ten day, was centrifuged (Micro 20, A. Hettich GmbH, Tutlingen, Germany) with 100 *g* (800 rpm) for platelet-rich plasma (PRP) or 2000 *g* (12500 rpm; Biofuge A, Haereus, Hanau, Germany) for platelet-poor plasma (PPP).

Platelet aggregation procedures are explicitly described in references 4, 9, and 10.

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-Alrich, Germany) (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 (*A*utomated *P*latelet *A*ggregation and *C*oagulation *T*racer, Biochemica GmbH, Flacht, Germany) 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, Konstanz, Germany), 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 monoacetylsalicylate (Bayer, Germany) 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. Drawing a line at the 50% value parallel to the x-axis yields an intersection with the aggregation curve. At this point, a perpendicular is raised from the x-axis and the IC_{50} value can be read off directly 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 in duplicate only, 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.

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