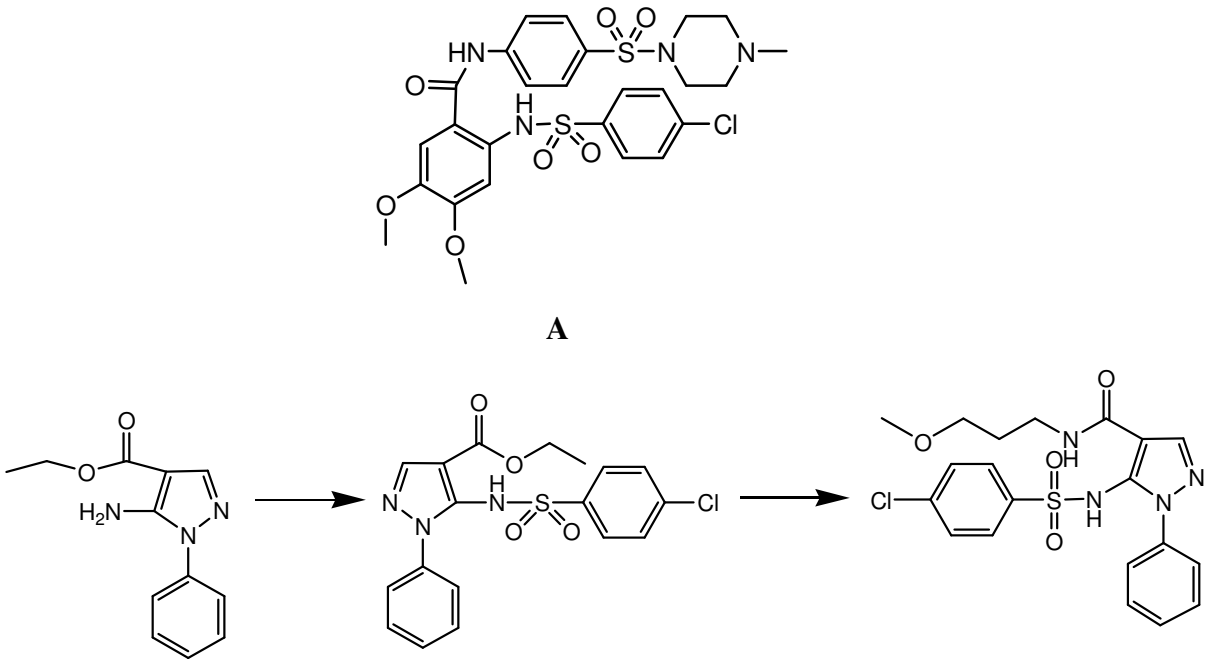
6 Summary

The aim of this work, was to synthesize new inhibiting agents of the phosphodiestrase 5 (PDE 5) or direct activators of the soluble guanylatcyclase (sGC).

According to the structures of **sildenafil** (Viagra®) a PDE 5-inhibitor and **YC-1** a direct activator of the soluble guanylatcyclase (sGC), the new substances were synthesized with a pyrazole-matrix and analyzed according to its physiological activity of clotting (see chapter 1, fig. 4 in page 5).

The starting material compounds got in this work was the 5-amino-1-phenyl-1H-pyrazol-4-carbonic acid ethyl ester **(1)** which in dependence to the substance **A** (sGC)-activator with 4-chlorphenylsulfonacidchloride sulfonates and subsequently implemented with different amines to the appropriate carbonic acid amides (type **3-13**). In fig. 34 the synthetic scheme of type **3-13** compounds is summarised.



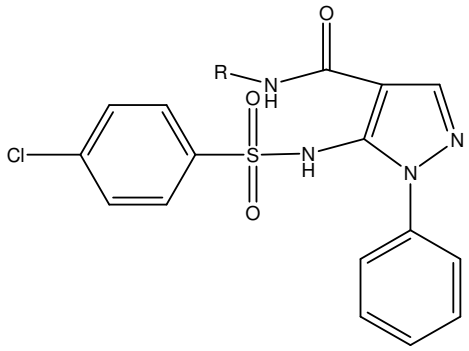
A

1 **2** **13b**

Fig. 34: structure comparison of substances 2 und **13b** with substance **A**

The results of the most active compounds in the Born test are summarised in chart 71.

Chart 71: half-maximum inhibiting concentration of the substances **4a, 4d, 7, 13a** and **13b**



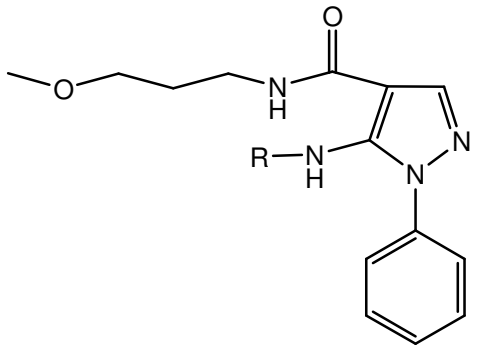
Nr.	R	IC_{50} [µmol/L]	
		4 min	20 min
4a	CH_3-NH-CH_2-CH_2-	>300	110
4d	C_4H_9 -NH- CH_2-CH_2-	>300	95
7	C_6H_{11}-NH- CH_2-CH_2- CH_2-	>300	28
13a	CH_3O-CH_2-CH_2-	249	105
13b	CH_3O -CH_2-CH_2-CH_2-	190	50

As the substance **13b** showed a moderate antithrombotic activity in the laser-thrombosis-model in arterioles, significant effects of inhibition of the thrombus generation (see chart 72, page 4) and in the Born-Test after 20 minutes of incubation time, the next step was to build the compounds of type **14-29**.

To get an accurate analysis of the effect of the substances, some of them were analyzed with more specific aggregation induces. The substance **7** displayed an IC_{50} value of 28 µmol/L when collagen was added after an incubation time of 20 minutes. After adding of PAF as aggregation elicitor a IC_{50} value of 0.45 nmol/L was detected. With **13b** the half maximum inhibitor concentration was 50 µmol/L (collagen). With adrenaline 5.8 nmol/L, which is caused by the effect of α_2 - receptor. With ADP was reduced to 540 nmol/L, which is caused by the effect of ADP-receptor.

Compound **4b** also shows good antiadrenergic and ADP-antagonistic attributes.
(IC_{50} = 94 nmol/L, in the ADP-induced aggregation and IC_{50} = 580 nmol/L on adrenaline induced aggregation). A very low half maximum inhibitor concentration of 120 nmol/L was achieved with the substance **9e**, when adrenaline was used as aggregation inducer.
ADP antagonistic attitudes were also detected in substance **4a** (IC_{50} = 500 nmol/L).
Then the substances which were examined *in vitro* in a Born-Test were examined *in vivo* in the laser-thrombosis-model to investigate their antithrombotic activity.
The substance **7** which showed the best antiaggregation activity after adding of collagen after 20min. of incubation time (IC_{50} = 28 µmol/L) and in addition shows very good PAF-antagonistic effects, surprisingly showed no effect in the laser-thrombosis-model. The best antithrombotic activities of were measured for the substance **13b**, **19b** and **27b**. The structural formula and the inhibition of thrombus formation of these three substances is summarized in chart 72.

Chart 72: inhibition of formation of thrombosis by these compounds **13b**, **19b** and **27b**



Nr.	R	Inhibition of formation of thrombosis			
		Venole		Arteriole	
		% ± s_x	α	% ± s_x	α
13b	4-Chlorphenylsulfonyl	2 ± 2	n.s.	**7 ± 2**	0.05
19b	Benzoyl	0 ± 1	n.s.	**6 ± 1**	0.05
27b	2-Naphthylsulfonyl	1 ± 1	n.s.	**7 ± 2**	0.02

On the basis of the structural formula of the substances **13b**, **21b** and **27b** the following structural elements can be made responsible for the antithrombotic activity:

• A methoxy group in the side chain, who´s distance to the amide function is three CH_2 -units.

• An electron withdrawing group at the exocyclic amino group in position 5 of the pyrazole ring, which carries a chlorine substituent in para-position of the aromaic ring of the benzoyl or sulfonyl group.

To examine , whether the antithrombotic activity of the substances is caused by the inhibition of the phosphodiesterase 5 or by the activation of the soluble guanylatcyclase (sGC), three structural different substances were chosen and analyzed by the Bayer company in two enzyme assays. From the tested substances none could inhibit the phosphodiesterase 5 (PDE-5) or activate the soluble guanylatcyclase (sGC). This shows, that the antithrombotic and/or antiaggregative activity of the analysed substances is not caused by the inhibition of PDE 5 or activation of the soluble guanylatcyclase.