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AN ATTEMPT TO SYNTHESIZE PROSTANOID THROMBOXANE A_2 ANTAGONISTS WITH AN ADDITIONAL PROSTACYCLIN LIKE QUALITY

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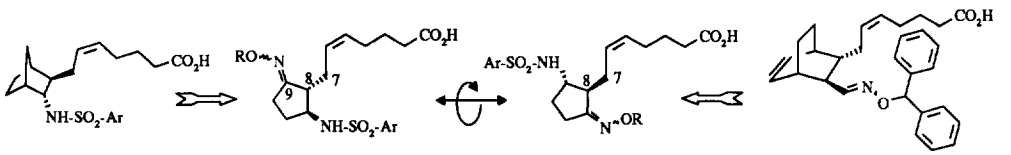
ABSTRACT: The preparation and biological characterisation of prostanoid TXA_2(TP)-antagonists which are modified at position 9 to add a PGI_2-like quality is described. Although we obtained highly potent TP-receptor antagonists, the synthesized compounds do not inhibit the ADP induced platelet aggregation and thus do not have any PGI_2(IP)-mimetic properties.

INTRODUCTION AND SYNTHESIS: Initially we were interested in the development of pure and stable TP-receptor antagonists for the combination with our potent IP mimetics Ilomedin®[1,2] and Cicaprost[3,4], since such a combination acts synergistically in the inhibition of platelet aggregation and should therefore increase the therapeutic index by reducing the required dose of IP and its mimetics[5].

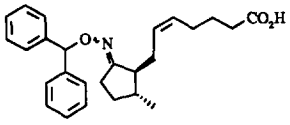
Another approach would be to combine the TP antagonistic and IP agonistic properties within one molecule. We were encouraged by results from Wilson and Jones who in 1985 described EP-157 (figure 1) which was designed to act as a TP-receptor antagonist but turned out to be a IP mimetic[6]. This unexpected result was attributed to the oximino ether moiety in EP-157 which was also used as the lower side chain in carbacyclins leading to compounds which bind to the PGI_2(IP)-receptor[7].

Previously[8] we prepared potent TP-receptor antagonists in the prostanoid series having aryl sulfonamides in the lower side chain. Because the biological potency seemed to be independent of the hybridisation in position 9 (9-fluoro or $\Delta^{8,9}$, respectively) we synthesized 9-oximino ethers in the 13-aza series. These compounds could be considered either as TP-antagonists comparable to S-145 or as a mimetic of the IP agonist EP-157 if -after a rotation around the C-C-bond at carbons 7 and 8 in **5**- the sulfonamide and the oxime ether moieties are formally exchanged as indicated below.



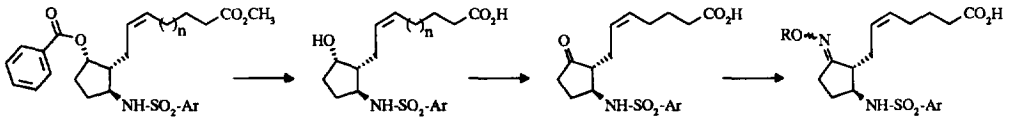
Dedicated to Professor Dr. Helmut Vorbrüggen on the occasion of his 65th birthday.

This idea was supported from the finding, that compound **1** binds to the TP receptor (C_F = 175) as well as to the IP receptor[9] (C_F = 25). Furthermore, the ADP-induced (1 µM) aggregation of human platelet rich plasma is inhibited with an IC_{50} of 0.8 µM.



1

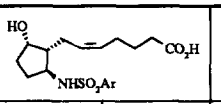
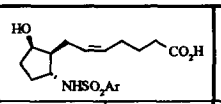
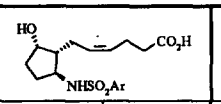
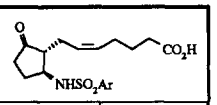
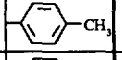
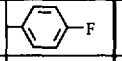
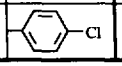
During prior work we had prepared the protected 9-hydroxy prostanoids **2** (n= 0,1)[8], which were now used as starting material. Saponification furnished the key compounds **3**, which after oxidation with Jones reagent to **4** and subsequent reaction with hydroxylamine ethers gave the 9-oximino ethers **5** as a nearly 1:1 mixture of the E/Z-isomers.



2 **3** **4** **5**

RESULTS AND DISCUSSION: Since the 9-hydroxy derivatives were available, we first evaluated the influence of the α-chain length as well as the absolute configuration on the biological activity of analogues of **3**. It turned out that the normal α-chain in combination with the natural absolute configuration leads to highly potent TP-receptor antagonists. All synthesized compounds do not inhibit the platelet aggregation induced by 1 µM ADP and thus do not have any PGI_2(IP)-mimetic properties. The corresponding 9-oxo analogues are equipotent. The substitution pattern of the sulfonamide moiety does not seem to play a major role (*table 1*).

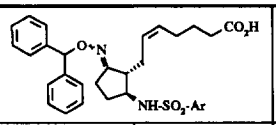
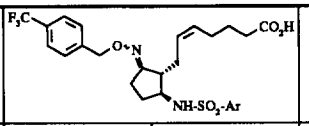
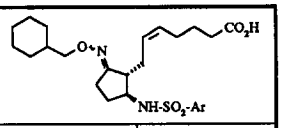
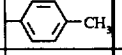
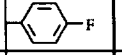
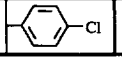
Table 1: Influence of the absolute configuration, the α-chain length and the hybridisation at carbon 9 on the TP-receptor binding and the inhibition of platelet aggregation.

table 1									
entry	**-Ar[10]**	**C_F**	**IC_{50}**	**C_F**	**IC_{50}**	**C_F**	**IC_{50}**	**C_F**	**IC_{50}**
1		7.0	0.50	340	5.8	135	2.5	6.5	0.67
2		2.1	0.64	26	6.0	200	1.6	6.4	0.50
3		0.68	0.19	85	2.2	31	1.8	2.6	0.64

Compared to the 9-oxo group, the diphenylmethyloximino ether does not affect the receptor binding but reduces the inhibition of platelet aggregation about 10-fold. Removing one phenyl group enhances the

receptor binding as well as the antiaggregatory potency. This effect is probably due to steric factors, as can be deduced from the corresponding analogues with a cyclohexylmethyloximino ether moiety which show comparable biological activities (*table 2*).

Table 2: Influence of the arylsulfonamide- and the oximino ether substitution pattern on the TP-receptor binding and the inhibition of platelet aggregation.

table 2							
entry	**-Ar**	C_F	IC_{50}	C_F	IC_{50}	C_F	IC_{50}
1		4.5	5.6	< 1.0	0.18	1.0	0.6
2		6.9	17	0.13	0.62	0.5	1.8
3		4.0	17	0.07	0.20	0.62	1.7

METHODS:

Measurement of thromboxane receptors on membranes of human thrombocytes

Blood from healthy human donors who denied having taken antiaggregatory medication, is taken into syringes containing ACD-buffer (3ml/20 ml blood). A centrifugation for 20 min at 160 g (1000 rpm) results in the separation of erythrocytes and leukocytes from platelet rich plasma (PRP). PRP is centrifuged at 800 g for 20 min (3600 rpm).

The platelet pellets are frozen in liquid nitrogen or solid carbondioxide/acetone and stored at - 80°C.

A pellet from 80 ml blood is homogenized with medium (140 mmol/l NaCl, 5 mmol/l KCl, 5 mmol/l glucose, 5 mmol/l citric acid, 2 mmol/l sodium citrate, 10 mmol/l tris, 1 mmol/l EDTA, 10 µmol/l indomethacin, pH 7.4) by ultrasound and in a potter type glass homogenizer. The homogenate is centrifuged (20 min at 105000g, 40000 rpm with the Ti 60 rotor). The pellet is resuspended in medium and recentrifuged. Finally the pellet is resuspended in 5 ml medium and stored in aliquots under liquid nitrogen. The protein content of this suspension is about 1 mg/ml. The above mentioned medium is also used for dilutions of agents and membranes.

75 µl ^3H SQ 29548[11] (1 pmol, 30 Ci/mmol), 50 µl competing substances in specified final concentrations and 75 µl membrane preparation are incubated at room temperature for 20 min. For comparison 2,5,10,20,30,50,100 nmol/l unlabelled SQ 29 548 is used as competitor. Nonspecific binding is defined as residual binding in the presence of 10 µmol/l unlabelled SQ 29548. Separation of tritiated SQ 29548 bound to membranes from that in solution is obtained by rapid filtration over GF/B filters followed by washing for 10 sec with isotonic saline (154 mmol/l NaCl, 10 mmol/l Tris/Cl pH 7.4). Total filtration and washing time does not exceed 10 sec. The filter bound radioactivity is counted after shaking the filters in scintillation fluid for at least 2 h.

Relative binding affinities of compounds are obtained by linearization of sigmoidal competition curves via a logit/log plot. Alternatively evaluation according to Munson and Rodbard with curve fitting procedures is performed. TP-receptor affinities are given as competition factors C_F = (IC_{50}-test)/(IC_{50}-standard); C_F - U46619 = 36. The K_D-value for the standard ^{3}H-SQ 29548 on platelet membranes is 20 nM.

Measurement of platelet aggregation

Platelet aggregation was assessed turbidimetrically in citrated human platelet rich plasma (PRP, treated with acetylsalicylic acid $5 \cdot 10^{-3}$ M and diluted 1:1 with Tyrode buffer), stirred continuously at 1000 rpm, where the optical density of unstimulated platelets was taken to represent 0% aggregation and that of platelet poor plasma to represent 100% aggregation. Test substances were added to PRP to investigate pro-aggregatory activity; in the absence of pro-aggregatory activity, the effects of test substance on aggregation induced by the stable TP-mimetic U 46619 (1 μM) were assessed following pre-incubation with test substance for 1 minute. All values are given in micromolar concentrations and represent a single determination.

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9. The IP-receptor affinity is given as competition factor C_F= (IC_{50}-test)/(IC_{50}-standard); in the binding assay ^{3}H-Iloprost is used as standard IP-receptor agonist at 10 nM. The K_D-value for ^{3}H-Iloprost on platelet membranes is 60 nM, while the IC_{50}-value in human platelet rich plasma is 1 nM.
10. The structures of all synthesized compounds were at least confirmed by high resolution NMR-spectroscopy.
11. Lefer A.M.; and Darius H. *Drugs Future* **1987**, 12, 367.

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