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NEW TXA_2/PGH_2 RECEPTOR ANTAGONISTS BASED ON THE CARBACYCLIN SKELETON

U. Klar* and A. Pletsch
Research Laboratories of Schering AG,
Müllerstr. 170-178, W-1000 Berlin 65, FRG

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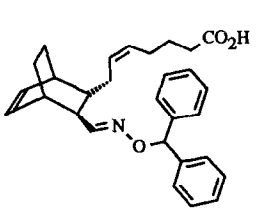
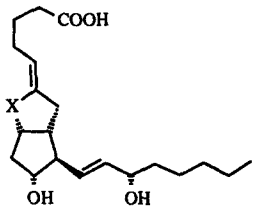
SUMMARY: Syntheses of novel TXA_2/PGH_2 receptor antagonists possessing the bicyclo[3.3.0]octane carbacyclin skeleton are described and structure activity relationships are discussed. It is shown that highly potent compounds can be obtained, when the lower prostanoid side chain is replaced by substituted aryl semicarbazones.

INTRODUCTION: Several years ago, Wilson and Jones succeeded in the synthesis of EP-157 (**I**) which was designed to act as a TXA_2/PGH_2 antagonist but turned out to be a PGI_2-mimic[1]. This unexpected profile was attributed to the oxime ether moiety which apparently changed the anticipated TXA_2/PGH_2 antagonist into a PGI_2-mimic[2].

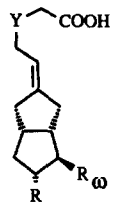
During our own search for specific and metabolically stable TXA_2/PGH_2-receptor antagonists[3], we also identified compounds which combine the desired TXA_2/PGH_2 antagonistic action with an additional, PGI_2-like quality, although these structures differ very strongly from that of PGI_2 (**II**, **X=O**), carbacyclin (**II**, **X=CH_2**) and their prostanoid analogues. We therefore concluded that it should be possible to convert a PGI_2 structure into a TXA_2/PGH_2 antagonist. For this purpose, we used a synthesis which offers high flexibility for the introduction of several lower side chains R_ω into the carbacyclin skeleton (**III**, **Y=CH_2**). To enhance the metabolic stability, we furthermore modified the natural α-chain either by removing one methylene group (**III**, **Y=bond**) or by introducing a 3-oxa moiety (**III**, **Y=O**) to slow down or even prevent β-oxidation, a common metabolic pathway for all prostanoids[4].



(±) **EP-157 (I)**



II

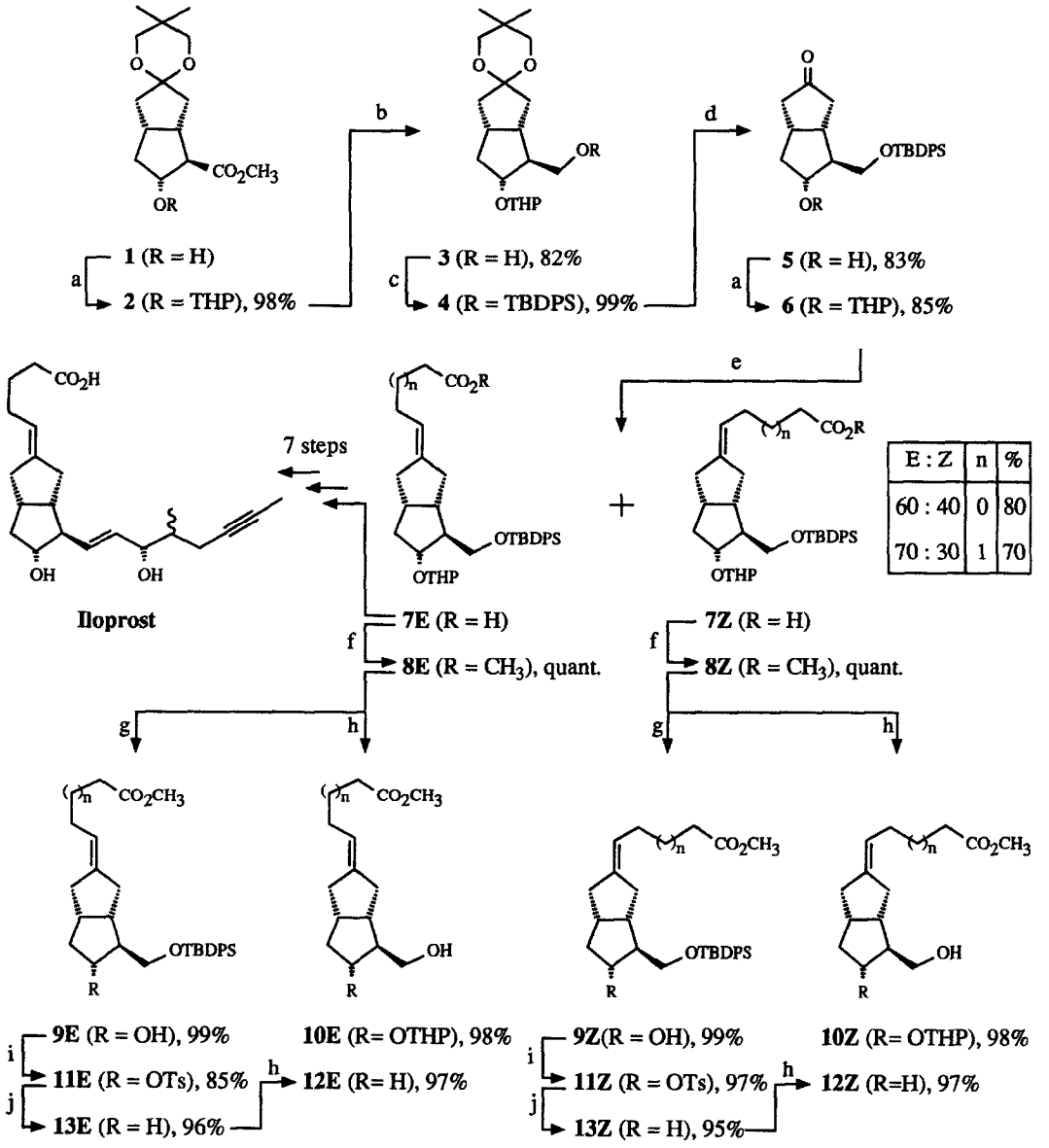


III

SYNTHESIS: The starting material **1**[5] (*scheme 1*) is readily available in optically pure form and is produced in large quantities during the synthesis of our stable carbacyclin analogue Iloprost. In a standard five step sequence (**1**→**6**), the functional groups are transformed and protected in a way suitable for our purpose. Compound **6** is the key intermediate to which different α-chains can be attached. As expected, Wittig-reaction with either carboxybutyl- or carboxypropyltriphenylphosphonium bromide gave mixtures of E/Z-isomers (**7**) which could be separated by repeated column chromatography on silica gel. To determine the configurations of the 5,6-double bonds unambiguously, we introduced the lower side chain of Iloprost to

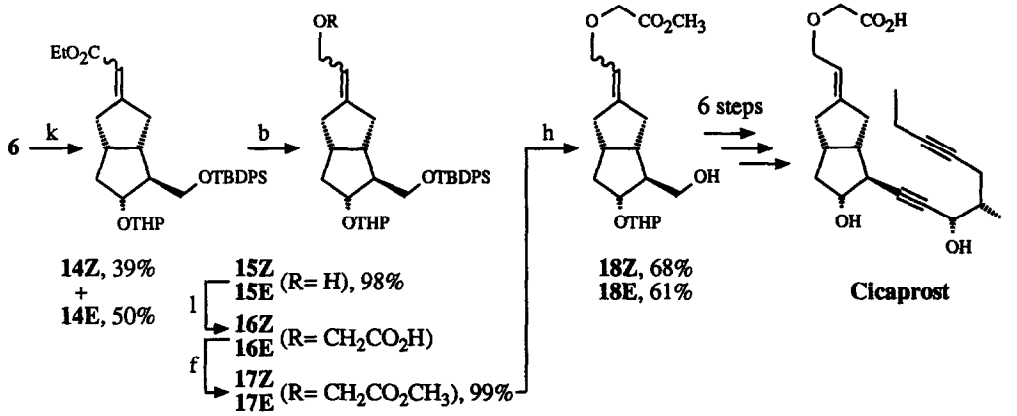
both isomers with the natural α-chain (n=1) and obtained two compounds, one of which was identical to **Iloprost** possessing the 5E configuration[6]. The intermediates **7E** and **7Z** with the truncated α-chain (n=0) were then assigned by comparing the polarities of the acids with their homologues. After esterification of the carboxylic acids, we cleaved either the silyl- or the THP-ether protecting groups. In the latter case (**9E**, **9Z**), the corresponding 11-tosylates were deoxygenated in high yields with $Zn/NaI/H_2O$ in DME[7].

Scheme 1



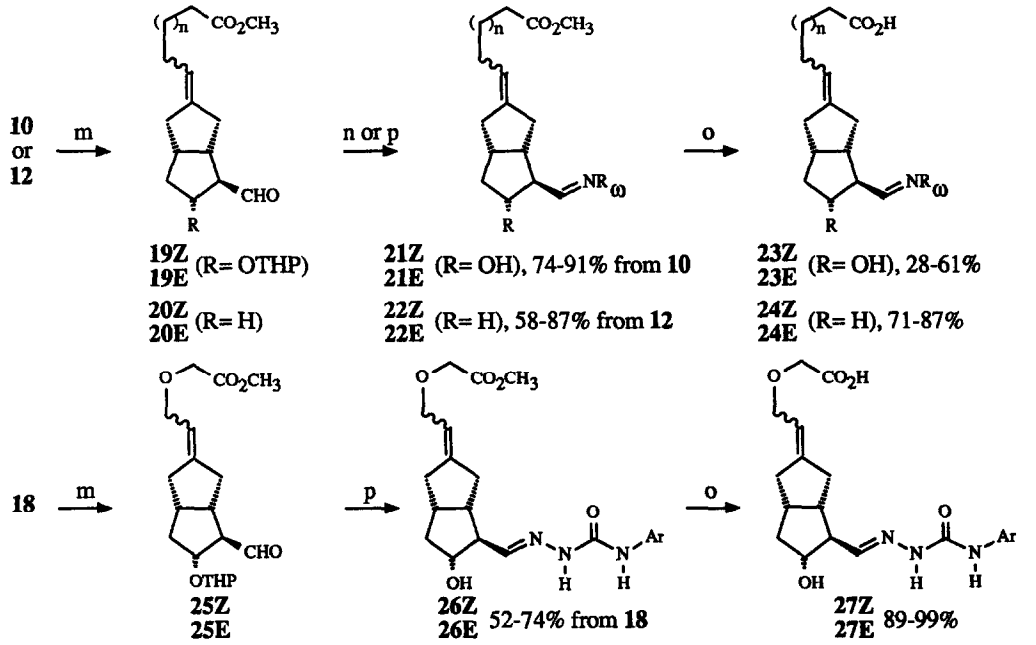
To establish a 3-oxa-moiety, we introduced a C-2 unit into **6** by a Wittig-Horner reaction (*scheme 2*). The E/Z isomers **14** were readily separated by chromatography on silica gel and reduced to the allylic alcohols **15E** and **15Z**. Subsequent etherification under phase-transfer conditions[8] then completed the synthesis of the α-chain. Again, the double bond configuration was assigned by the synthesis of **Cicaprost** and its 5Z-isomer as reference compounds[8].

Scheme 2



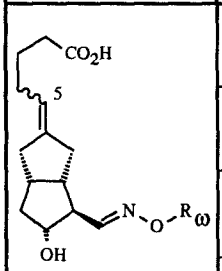
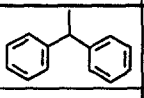
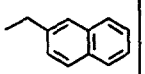
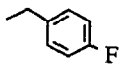
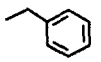
Having all the intermediates with different α-chains and defined double bond configuration available, we now synthesized some oxime ethers in analogy to Wilson and Jones. Furthermore, we made several aryl semicarbazones (compare the Squibb compounds[9], e.g., SQ 29548[10]) from which we expected that they would add a TXA_2/PGH_2-antagonistic quality to the carbacyclin skeleton (*scheme 3*).

Scheme 3



CONDITIONS: **a:** dihydropyran, cat. p-TsOH, CH_2Cl_2, rt, 1h; SiO_2; **b:** DIBAL, toluene, 0°C, 0.5h; **c:** TBDPS-Cl, imidazole, DMF, rt, 16h; SiO_2; **d:** HOAc, H_2O, THF, rt, 16h; SiO_2; **e:** Li-HMDS, carboxybutyl- or carboxypropyltriphenylphosphonium bromide, THF, 30°C-60°C, 2-6h; SiO_2; **f:** CH_2N_2, ether, 5°C, 0.1-1h; SiO_2; **g:** cat. PPTs, EtOH, 55°C, 1-4h; SiO_2; **h:** 1M TBAF, THF, rt, 2-4h; SiO_2; **i:** TsCl, pyridine, 50°C, 2h; SiO_2; **j:** Zn, NaI, H_2O, DME, rf, 15h; SiO_2; **k:** Ph_3P=CH-CO_2Et, toluene, 80°C, 1-7d; SiO_2; **l:** Br-CH_2CO_2-^{t}Bu, 50% KOH, cat. Bu_4NHSO_4, 2.5h; SiO_2; **m:** $C_2O_2Cl_2$, DMSO, CH_2Cl_2, -65°C→-40°C, 2.5h; Et_3N; **n:** H_2N-O-Ar, PPTs, EtOH, 55°C, 3-18h; SiO_2; **o:** 5% LiOH, MeOH, rt, 2-16h; SiO_2; **p:** arylsemicarbazide hydrochloride, 1eq pyridine, EtOH, 55°C, 3-18h; SiO_2.

RESULTS AND DISCUSSION: As expected, the introduction of the diphenylmethyl oxime ether moiety of EP-157 into the carbacyclin skeleton (*table 1*, entry 1) led to a pair of compounds of which the E-configured isomer shows an affinity to the IP-receptor, although the binding is 160 times weaker compared to Iloprost. More interestingly, both isomers bind to the TP-receptor, so the oxime ether element of EP 157 can introduce PGI_2-agonistic as well as TXA_2/PGH_2-antagonistic qualities. Modifying the benzylic substituent, we could improve the binding to the IP-receptor even in those compounds, which have the "wrong" 5Z configuration for the IP-receptor. It is also interesting to note, that while the higher affinity to the IP-receptor is observed for the 5E-isomers, the TP-receptor affinity is emphasized for the 5Z-isomers (*table 1*, entries 2 to 4).

Table 1	entry	R_ω	$\Delta^{5,6}$	receptor affinities *		inhibition of blood platelet aggregation induced by		
				C_F TP	C_F IP	ADP (IC_{50})×10^{-6}M	10^{-6}M U 46619 (IC_{50})×10^{-6}M	potency relative to SQ 29548
 **23**	1		E	95	160	1.0	1.6	0.03
			Z	210	nc	-	23.0	0.001
	2		E	280	31	1.1	1.6	0.03
			Z	90	76	-	2.2	0.002
	3		E	>1000	20	1.5	1.9	0.009
			Z	500	250	-	14.0	0.001
	4		E	>1000	7	2.0	1.9	0.009
			Z	470	100	>50	5.4	0.001

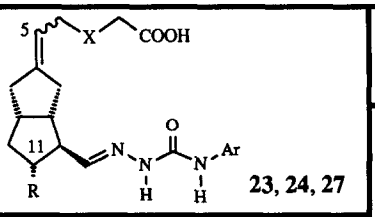
*: The TP- and IP-receptor affinities in *tables 1* and *2* are given as competition factors C_F=(IC_{50}-test)/(IC_{50}-standard); nc means no competition;
SQ 29548 is used as standard TP-receptor antagonist, Iloprost as standard IP-agonist.

Turning to aryl semicarbazones as ω-chains, we obtained compounds which are potent and pure TP-receptor antagonists. Without any exception, the 5Z-isomers are more active in inhibiting the aggregation of human platelet-rich plasma (induced by the standard TP-receptor agonist U 46619) compared to their 5E-isomers. Since none of the compounds tested showed a better affinity to the IP-receptor than 1/400 compared to Iloprost, PGI_2-quality is generally minute or lost.

Removal of the 11-hydroxy group (*table 2*, compare entries a and b) lowers TP-receptor affinity by a factor of 2 to 16, while antiaggregatory potency (relative to SQ 29548) is even more reduced by a factor of 10 to 330.

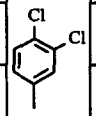
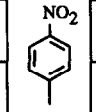
Shortening the natural α-chain by one methylene group (compare entries a and c) also lowers TP-receptor affinities by factors of 5 to 12 for the Z-isomers and 20 to 30 for the E-isomers. Similar observations can be made for the antiaggregatory potency (2 to 4 fold reduction for the Z-isomers and a 4 to 25 fold one for the E-isomers, respectively). One remarkable exception is given by the nitro compound of entry 4c/Z which is 50 times more active than the parent compound (entry 1a/Z).

When the methylene group at C-3 is substituted by an oxygen atom (compare entries a and d), TP-receptor affinity is also reduced 4 to 20 fold. Interestingly, the change in antiaggregatory potency is not so clear in this case. While some compounds show a significantly reduced activity (entries 1d/Z, 2d and 3d/Z), other derivatives are even more potent (entries 3d/E and 4d).



23, 24, 27

Table 2

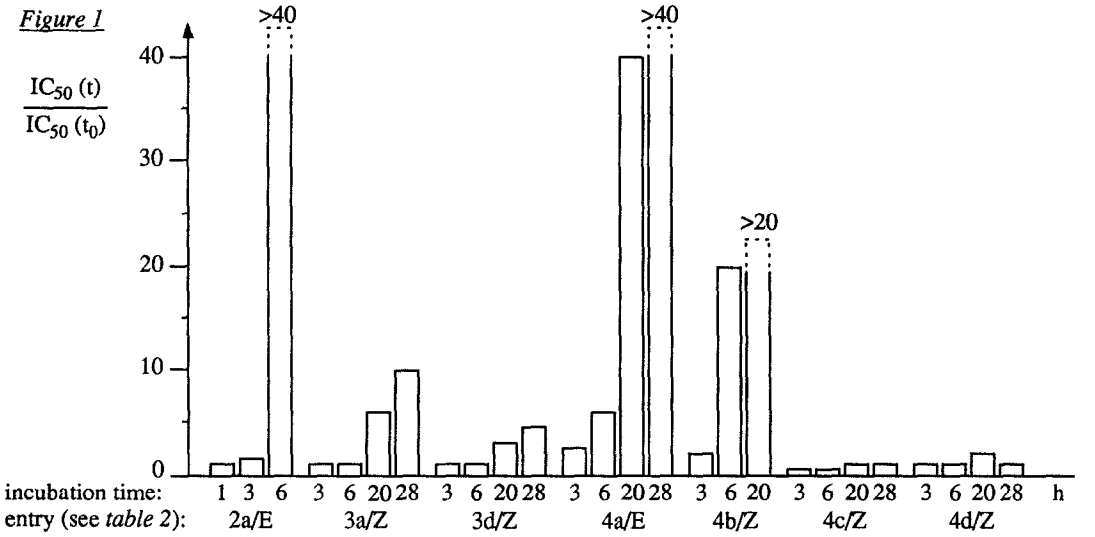
X	R	Entry No.[5]Δ	Ar	C_F TP	(IC_{50}) $\times 10^{-6}$M	potency relative to SQ 29548	Entry No. [5]Δ	Ar	C_F TP	(IC_{50}) $\times 10^{-6}$M	potency relative to SQ 29548
				inhibition of blood platelet aggregation of human platelet-rich plasma induced by 10^{-6}M U46619							
CH_2	OH	1a E		20.0	1.7	0.005	3a E		0.8	0.2	0.038
		Z		1.5	0.1	0.17	**Z**		**0.1**	**0.02**	**1.0**
CH_2	H	1b E		40.0	12.0	0.0004	3b E		4.3	9.0	0.002
		Z		3.0	2.3	0.002	Z		1.6	1.8	0.003
bond	OH	1c E		454.0	26.0	0.0007	3c E		16.0	1.7	0.008
		Z		10.0	0.6	0.059	Z		0.8	0.19	0.33
O	OH	1d E		138.0	6.0	0.008	3d E		2.9	0.18	0.11
		Z		16.0	1.4	0.014	Z		0.7	0.06	0.33
CH_2	OH	2a E		1.6	0.13	0.09	4a E		0.7	0.06	0.1
		Z		0.2	0.06	0.25	Z		0.1	0.05	0.33
CH_2	H	2b E		16.0	6.5	0.0007	4b E		2.2	1.7	0.01
		Z					Z		0.7	0.58	0.01
bond	OH	2c E		50.0	5.2	0.004	4c E		22.0	0.52	0.027
		Z		2.4	0.6	0.11	**Z**		**0.5**	**0.02**	**8.3**
O	OH	2d E		18.0	1.6	0.03	4d E		9.4	0.19	0.33
		Z		4.0	0.18	0.11	**Z**		**0.9**	**0.02**	**1.0**

The absolute potency is also strongly influenced by the substitution pattern of the phenyl group. The introduction of one chlorine atom in the para (data not shown) or meta position enhances the TP-receptor binding as well as the antiaggregatory potency 2 to 20 fold, whereas a chlorine atom located in the ortho position (data not shown) has only a weak influence. In general, a further enhancement of activity (2 to 6 fold) can be observed by introducing two chlorine atoms in the 3,4-positions.

The introduction of a para-nitro group leads to the strongest enhancement in antiaggregatory potency (2 to 140 fold) compared with the unsubstituted analogues. The Z-isomers of the compounds in entries 3a and 4d are equipotent and that of entry 4c is 8 times more active *in vitro* than the standard TP-receptor antagonist SQ 29548!

To estimate the metabolic stability, seven compounds from *table 2* were incubated with cultured rat liver cells and the remaining antiaggregatory activity ($IC_{50}(t)$) was determined 1, 3, 6, 20 and 28 hours after incubation (*figure 1*). This preliminary test shows that metabolically more stable compounds can be obtained either by removing one methylene from or by introducing a 3-oxa moiety into the natural α-chain (compare *figure 1*, 4a-d). Compound 2a/E having a 3-chlorophenyl in the ω-chain is metabolized much faster compared with the derivatives possessing a 3,4-dichloro- (3a/Z) or a 4-nitrophenyl group (4a/E). Whether the 5,6-double bond configuration has an additional influence on the metabolism cannot be estimated from these data.

Figure 1



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