Guanidine Derivatives as Combined Thromboxane A_2 Receptor Antagonists and Synthase Inhibitors

Rainer Soyka,* Brian D. Guth, Hans M. Weisenberger, Peter Luger,† and Thomas H. Müller‡

Research and Development, Boehringer Ingelheim Pharma KG, Birkendorfer Strasse 65, 88397 Biberach, Germany, Free University of Berlin, Institute for Crystallography, Takustrasse 6, 14195 Berlin, Germany, and Institute Oldenburg, German Red Cross Transfusion Service, Brandenburger Strasse 21, 26133 Oldenburg, Germany

Received December 9, 1998

A new series of ω-disubstituted alkenoic acid derivatives derived from samixogrel **5** were designed and synthesized as combined thromboxane A_2 receptor antagonists/thromboxane A_2 synthase inhibitors with improved solubility and reduced protein binding compared to **5**. Hexenoic acid derivatives with a 3-pyridyl group and 3-(2-cyano-3-alkyl-guanidino)phenyl substituent were found to be optimal with regard to this dual mode of action. The most potent compound, *E*-6-(3-(2-cyano-3-*tert*-butyl-guanidino)phenyl)-6-(3-pyridyl)hex-5-enoic acid, "terbogrel" **32** inhibits the thromboxane A_2 synthase in human gel-filtered platelets with an IC_{50} value of 4.0 ± 0.5 nM ($n = 4$). Radioligand binding studies with ^{3}H-SQ 29,548 revealed that **32** blocks the thromboxane A_2/endoperoxide receptor on washed human platelets with an IC_{50} of 11 ± 6 nM ($n = 2$) and with an IC_{50} of 38 ± 1 nM ($n = 15$) in platelet-rich plasma. Terbogrel inhibits the collagen-induced platelet aggregation in human platelet-rich plasma and whole blood with an IC_{50} of 310 ± 18 nM ($n = 8$) and 52 ± 20 nM ($n = 6$), respectively. This was shown to translate into a potent antithrombotic effect in vivo as demonstrated in studies using a model of arterial thrombosis in rabbits ($ED_{50} = 0.19 \pm 0.07$ mg/kg; $n = 20$). Thus, terbogrel is the first compound with a guanidino moiety demonstrating both a potent TXA_2 synthase inhibition and a potent TXA_2 receptor antagonism and has been selected for further clinical development.

The importance of thromboxane A_2 (TXA_2) **1** (Chart 1) in coronary thrombotic syndromes is supported by the antithrombotic effects of TXA_2 inhibitors in various animal studies[1−4] as well as indirectly by the proven clinical efficacy of aspirin in the secondary prevention of myocardial infarction.[5] Indeed, despite the therapeutic efficacy of aspirin in various thrombotic syndromes, which has been attributed exclusively to its thromboxane inhibiting effect,[6] aspirin is optimal neither in terms of its efficacy nor side effect profile.[7−9]

Both the inhibition of TXA_2 synthase (TxSI) and the selective blockade of TXA_2 receptors (TxRA) have been pursued as alternative therapeutic strategies to prevent the prothrombotic action of TXA_2 without blocking cyclooxygenase activity, as does aspirin. Selective blockade of TXA_2 receptors does not interfere with arachidonic acid metabolism in activated platelets, but it does prevent the activation of platelets by the binding of TXA_2 or endoperoxides (endo) to a common membrane receptor (TXA_2/endo) on the human platelet. Additionally, on smooth muscle cells, TXA_2/endo receptor blockade prevents the vasoconstrictive action of TXA_2. While a theoretically attractive approach, a high level of receptor blockade may be required to produce clinically relevant effects, thereby placing great demands on both the pharmacodynamic and pharmacokinetic characteristics of such a blocking agent. Alternatively, the selective inhibition of thromboxane synthase prevents the conversion of prostaglandin endoperoxide intermediates (PGH_2, PGG_2) to TXA_2. This has the advantage that other arachidonic acid metabolites can still be produced.

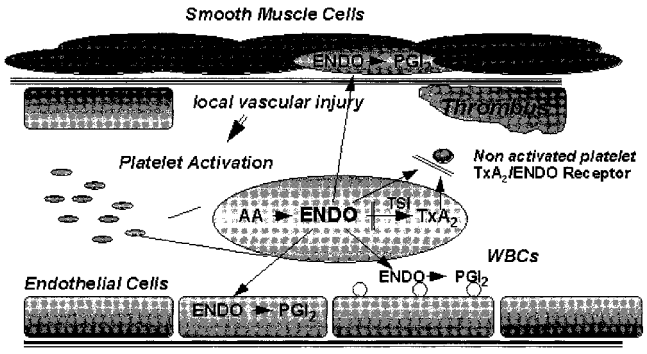


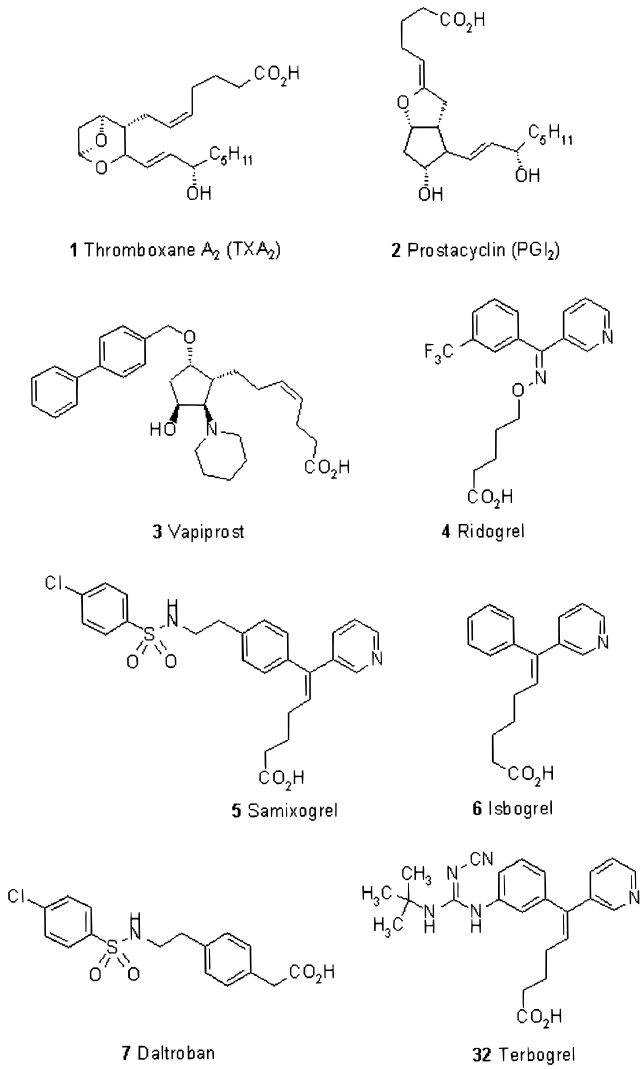
Figure 1. Schematic diagram of the antithrombotic effect of combined TxA_2/endo receptor blockade and thromboxane synthase inhibition (TSI). Endoperoxides (ENDO) produced from arachidonic acid (AA) in platelets can be metabolized to prostacyclin (PGI_2) in smooth muscle cells, endothelial cells, and white blood cells (WBCs). The potential prothrombotic effect of the endoperoxides is prevented through TxA_2/endo receptor blockade.

Thus, in the setting of platelet activation, as would occur locally at a vascular injury site (Figure 1), the platelet-produced endoperoxides can be taken up by other cells (smooth muscle cells, endothelial cells, and white blood cells) which can then produce prostaglandin I_2 (prostacyclin) **2** and other antithrombotic prostanoids.[10,11] Prostacyclin is of particular interest since it is one of the most effective inhibitors of platelet activation known and it is a potent vasodilator. Therefore, with a thromboxane synthesis inhibitor the antithrombotic effect due to the presence of locally produced prostacyclin could exceed that expected by only blocking TXA_2 receptors. Additionally, the conversion of the endoperoxides to

† Free University of Berlin.
‡ Institute Oldenburg.

10.1021/jm9707941 CCC: $18.00
Published on Web 03/23/1999

Chart 1



E-type prostaglandins could help to reduce thrombus formation due to their vasodilatory action. Finally, the recently recognized leucocyte inhibiting activity of *E*-type prostaglandins could help to reduce any ischemic damage, which may occur distal to an obstructive thrombus.[12] This is an additional, potentially therapeutic, effect not produced by prostacyclin.

The clinical experience using these two pharmacological strategies alone to inhibit TXA_2 has been disappointing, however. For example, the first large clinical trial with the selective and potent TXA_2 receptor antagonist Vapiprost **3** in PTCA patients showed no effect on restenosis 6 months following the procedure.[13] The clinical experience with thromboxane synthase inhibition has not been better. The recent RAPT study with Ridogrel **4**, a potent TXA_2 synthase inhibitor and weak receptor antagonist, did not significantly affect the primary endpoint of infarct-related artery patency at 2 weeks following streptokinase-induced lysis in acute myocardial infarction patients.[14] Various other smaller studies have provided mixed results with these two types of TXA_2-blocking agents.[15−17]

A possible explanation of the only modest clinical success with TXA_2 synthase inhibition comes with the recognition that the endoperoxide intermediates produced by cyclooxygenase in activated platelets bind and activate the same membrane receptor as does TXA_2 thereby causing platelet aggregation.[18] If thromboxane synthase inhibition leads to locally high concentrations of endoperoxides as hypothesized, these metabolites could, at least in part, take over the thrombogenic role of TXA_2.

The apparent solution to optimize therapeutic benefit is to combine thromboxane synthase inhibition with TXA_2/endo receptor blockade.[19,20] With such combined treatment, the prostaglandin endoperoxides are synthesized in activated platelets but their prothrombotic effect is inhibited by the simultaneous TXA_2/endo receptor blockade (Figure 1). The endoperoxides can, however, be metabolized by other cell types to produce antithrombotic prostanoids (e.g., prostacyclin and PGE_1/PGE_2) which should provide a potent, local antithrombotic and vasodilatory action. A variety of compounds with both properties have been reported.[21] Ridogrel was the first putative dual inhibitor of TXA_2 to be tested clinically, but as mentioned above, it yielded disappointing clinical results. Evaluation of the pharmacological characteristics of ridogrel[22] would suggest, however, that the receptor affinity of the compound is indeed too weak to adequately qualify as a compound with a dual mode of action. In continuation of our previous work[23] we looked for new compounds with strong and balanced TXA_2 receptor blockade and synthase inhibition.

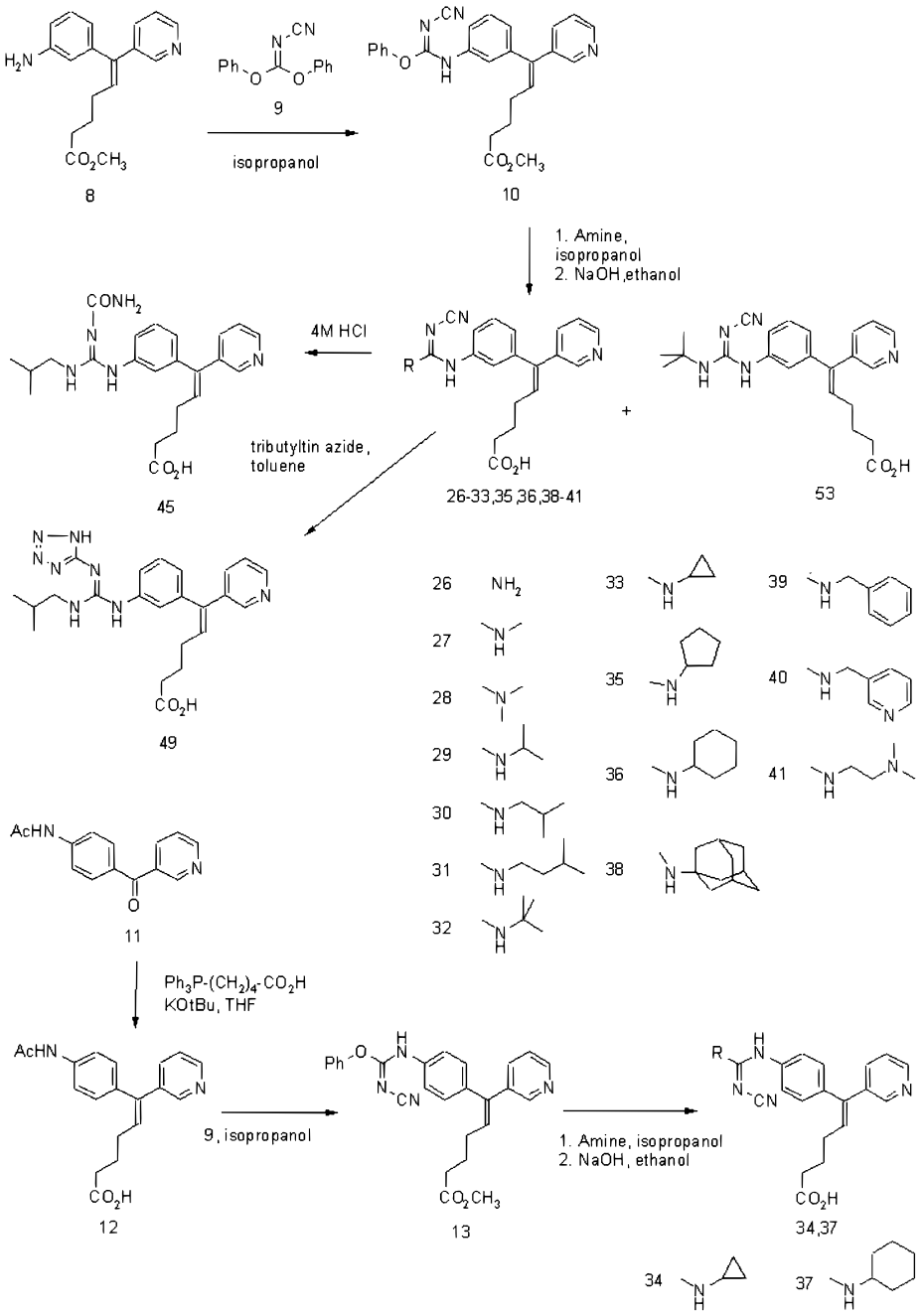
Compound Design

In our previous publication[23] we reported the synthesis and biological properties of 6,6-disubstituted hex-5-enoic acid derivatives as combined TXA_2 synthase inhibitors and receptor antagonists. The most interesting molecule in this series was compound **5** (INN: samixogrel) which reflects structural elements of the synthase inhibitor isbogrel **6** and the receptor antagonist daltroban **7**.

TXA_2 synthase from human platelets is a cytochrome P450 enzyme that contains one heme per polypeptide chain.[24] Essential structural features of TxSIs are a basic nitrogen atom of a 3-substituted pyridine or an N-substituted imidazole ring and a carboxylic acid group separated by a distance of 0.85−1 nm.[25] It was postulated that the pyridine or imidazole moiety forms a complex via the nitrogen atom with the heme group of the catalytic site of TXA_2 synthase.[26] Assuming a staggered conformation for the carboxyalkyl chain of **5**, the distance between the carbon atom of the carboxylic acid and the nitrogen atom of the pyridine ring fulfills the above-mentioned requirement.

Unfortunately compound **5** showed only moderate plasma levels combined with an unexpected high variability after oral administration in human volunteers, presumably due to the very low solubility of this molecule in aqueous solutions under physiological conditions. Due to the fact that the *E*-6-(3-pyridyl)-6-phenyl-hex-5-enoic acid element is essential for potent TXA_2 synthase inhibition, we concentrated on the phenylsulfonamide group of **5.** Addition of different substituents to the *p*-chlorophenyl ring and the nitrogen atom of the sulfonamide with the aim to increase solubility in aqueous solution was, however, not suc-

Scheme 1



cessful. On the other hand we knew from our own investigations[23] that the *p*-Cl-phenylsulfonamide moiety of samixogrel **5** could be directly connected to the 6-phenyl-hexenoic acid part preferentially in the meta position without loss of activity. Unfortunately such compounds revealed an unexpectedly strong unspecific protein binding with concomitant reduction of antiplatelet activity in the presence of plasma proteins. Because it was known from the literature[21] that the sulfonamide moiety is not a prerequisite for TXA_2 receptor antagonism, we looked for structural elements with steric and electronic properties similar to phenylsulfonamides. Starting with simple urea and thiourea derivatives, we found that nonbasic guanidines were suitable substitutes for the sulfonamide group. Due to synthetic considerations (see Chemistry section) the *p*-chlorophenyl ring of **5** had to be replaced by alkyl groups.

Chemistry

The compounds described in Tables 1−3 were prepared as depicted in Schemes 1−4. Compound **10** was synthesized (Scheme 1) by reaction of compound **8**[23] with commercially available diphenyl-*N*-cyano-carbonimidate **9** in ^{i}PrOH at ambient temperature. Starting from **10**, compounds **26**−**33, 35, 36, 38**−**41** were obtained by reaction with the corresponding amines in refluxing ^{i}PrOH followed by saponification with aqueous NaOH in moderate to good yields. Usage of commercially available *N*-cyano-dithiocarbonimidic acid dimethyl ester instead of **9** with the same protocol gave only poor yields, especially in the substitution reaction of the second methylthio group. Whereas aliphatic amines, even if they were sterically hindered, reacted easily with **10**, anilines could not be transformed to the

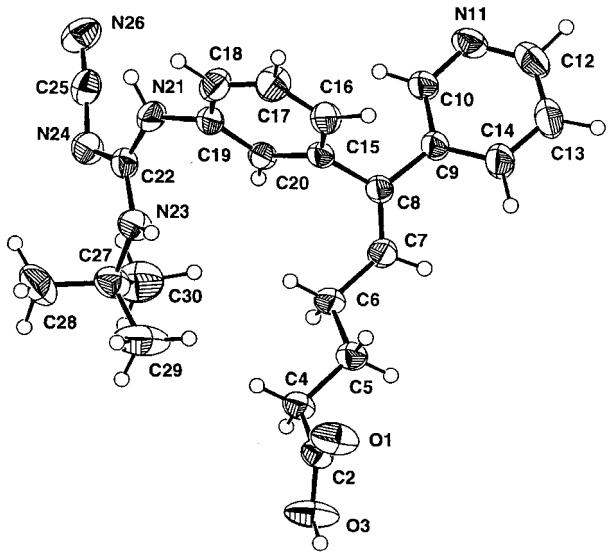


Figure 2. X-ray structure of compound **32**·0.5CH_3COCH_3 acetone (ORTEP[37]-plot).

corresponding cyano-guanidines. The *E*-configuration of the C−C double bond was generally confirmed by ^{1}H NMR spectroscopy and by X-ray analysis for compound **32** (Figure 2). Concerning the C−N double bond we could not detect isomers at room temperature for any compound in Table 1 by ^{1}H NMR spectroscopy, presumably due to the more formal character of this double bond. This explanation is in accordance with the X-ray analysis of **32** which shows that the bond length of the C−N double bond (C22−N24: 132.9 ± 0.5 pm) does not differ substantially from the other C−N bonds (C22−N23: 132.9 ± 0.4 pm; C22−N21: 136.0 ± 0.4 pm) of the cyano-guanidine moiety. This bond geometry compares well with the unsubstituted cyano-guanidine.[27]

Repeated column chromatography of the mother liquid after recrystallization of **32** gave the *Z*-isomer **53**. The regioisomers **34** and **37** were prepared from the ketone **11**,[23] which was transformed to **12** by a Wittig reaction via the intermediate **13** according to the above-mentioned protocol.

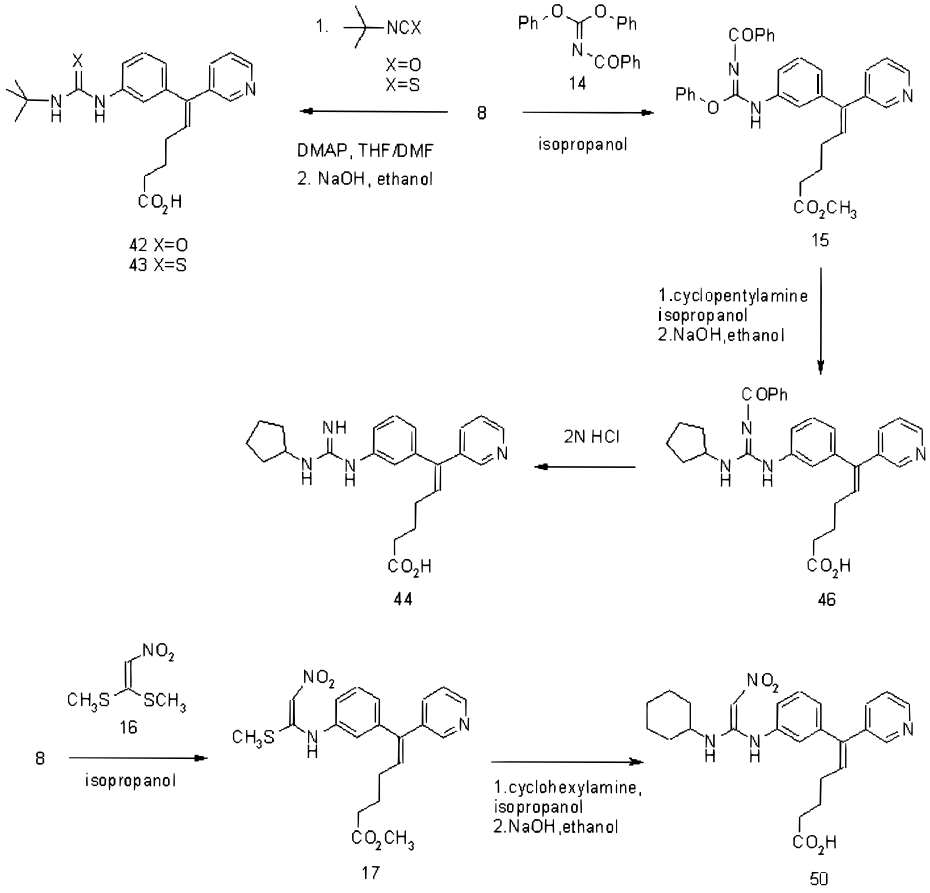
Acidic hydrolysis of the *N*-cyanoguanidine **30** resulted in the *N*-carbamoyl derivative **45**, and 1,3-dipolar cycloaddition with tributyltin azide gave the *N*-tetrazolyl compound **49** in a yield of 64%.

The urea and thiourea derivatives **42** and **43** (Scheme 2) were prepared by reaction of **8** with *tert*-butylisocyanate and *tert*-butylisothiocyanat in THF/DMF in the presence of DMAP. Whereas **42** could be prepared in 52% yield, the reaction with the corresponding isothiocyanate gave **43** only in 10% yield after refluxing the reactants for more than 40 h.

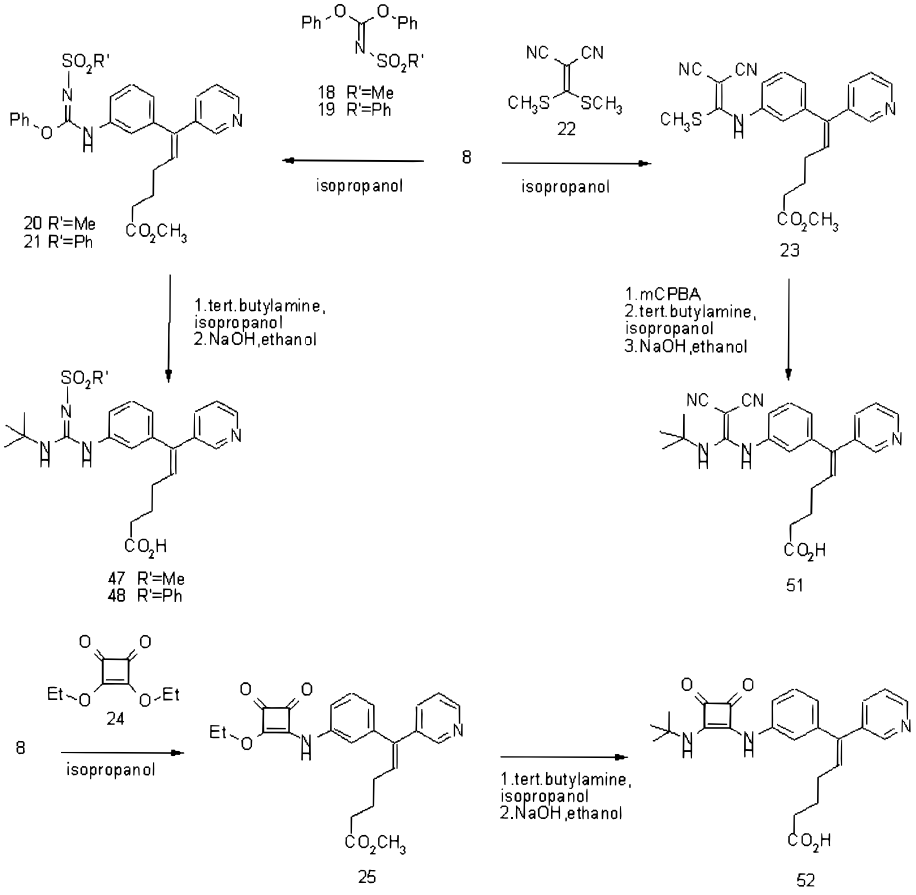
Condensation of **8** with *N*-diphenoxymethylene-benzamide **14**[28] at room temperature gave compound **15** which was further transformed to **46** by reaction with the cyclopentylamine in refluxing ^{i}PrOH. Compound **44** was obtained from **46** by cleavage of the benzoyl group in refluxing HCl. Compound **50** was prepared by a similar protocol via the intermediate **17** starting from **8** and commercially available 1,1-bis(methylthio)-2-nitroethylene **16**.

The sulfonyl derivatives **47** and **48** (Scheme 3) were prepared via the intermediates **20** and **21** which were obtained by reaction of **8** with *N*-diphenoxymethylene-

Scheme 2



Scheme 3



methanesulfonamide **18**[28] and *N*-diphenoxymethylene-phenylsulfonamide **19**. Compound **19** was obtained by treatment of diphenoxy-dichloromethane with phenylsulfonamide in 26% yield.

Condensation of **8** with (bis-methylsulfanyl-methylene)malononitrile **22**[29] yielded compound **23**. Direct substitution of the methylthio group with *tert*-butylamine in refluxing ^{i}PrOH was not successful, but after oxidation with mCPBA compound **51** could be obtained in 43% yield. The squaric acid derivative **52** could be obtained by reaction of **8** with 3,4-diethoxy-3-cyclobutene-1,2-dione **24** to form compound **25** which was then treated with *tert*-butylamine followed by saponification of the methyl ester.

Reaction of the ketone **54**[23] with the corresponding carboxyalkyl-triphenylphoshonium bromides yielded the compounds **55**−**57** which were then treated with HCl in MeOH to form the methyl esters with simultaneous cleavage of the acetyl moiety. Reaction with reagent **9** and then with *tert*-butylamine followed by cleavage of the methyl ester under basic conditions resulted in the compounds **58**−**60**. The *E*-configuration of the C−C double bond was confirmed by ^{1}H NMR spectroscopy.

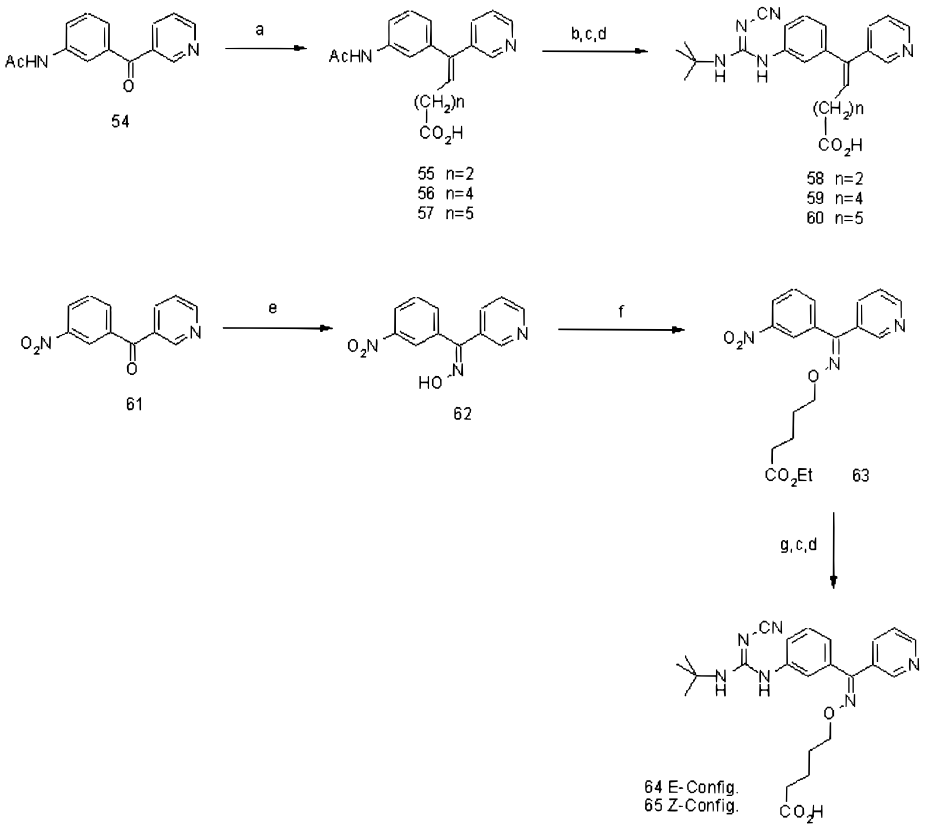
Reaction of the ketone **61**[30] with hydroxylamine gave the oxime **62** as a mixture of *E*- and *Z*-isomers, which was further treated with ethyl 5-bromopentanoate to form compound **63**. After reduction of the nitro group, reaction with **9** and then *tert*-butylamine followed by cleavage of the ethyl ester and separation of the *E*- and *Z*-isomers by column chromatography yielded the oxime ether derivatives **64** and **65**.

In Vitro Pharmacology

The compounds described above were initially tested in the following three assays: (a) displacement of the high-affinity radiolabeled ligand ^{3}H-SQ 29,548 from the PGH_2/TXA_2 receptor on human gel-filtered platelets, (b) inhibition of TXB_2 formation by human gel-filtered platelets incubated with ^{14}C-arachidonic acid, and (c) inhibition of collagen-induced platelet aggregation in human platelet-rich plasma (PRP).

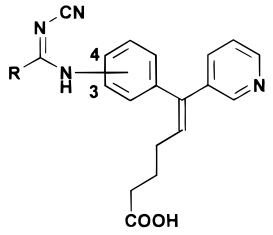
The results are listed as IC_{50}/EC_{50} values in Tables 1−3. Starting from the *N*-cyano-guanidine derivatives, we first examined the influence of the alkyl substituent on both properties, TXA_2 receptor antagonism and synthase inhibition. It is remarkable that already the unsubstituted derivative **26** exhibits potent antagonistic activity whereas the synthase inhibitory potency is weak compared to samixogrel **5** or ridogrel **4**. Monoalkylation of the terminal nitrogen atom of the *N*-cyano-guanidine moiety (**27, 29**−**39**) improves the enzyme inhibition by a factor of 100, but there is no strong relationship between the bulkiness and substitution pattern of the alkyl groups and synthase inhibition. Besides aromatic systems (**39**), also alicycles from cyclopropyl (**33**) up to cyclohexyl (**36**) as well as the very bulky group adamantyl (**38**) were tolerated. The aliphatic part of the compounds seems nonessential for the interaction with the enzyme, but to inhibit the TXA_2 synthase the compounds have to penetrate the cell membrane because the enzyme is located within the mitochondria. An increase in lipophilicity presumably facilitates the

Scheme 4[a]



[a] (a) Ph_3P-$(CH_2)_m$-COOH (m = 3, 5, 6), KOtBu, THF; (b) HCl, MeOH; (c) **9**, ^{i}PrOH; (d) (1)*tert*-butylamine, ^{i}PrOH, (2) NaOH, EtOH; (e) $H_2NOH{\cdot}HCl$, pyridine, EtOH; (f) Br-$(CH_2)_4$-COOEt, KOtBu, DMF; (g) Ra−Ni, H_2, EtOH.

Table 1. Physical Constants and in Vitro Activity of Compounds[a]

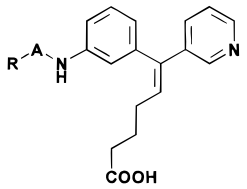


no.	R	pos.	formula	mp [°C]	TXA_2 receptor antagonism IC_{50} [μM]	TXA_2 synthase inhibition IC_{50} [μM]	Inh. of collagen-induced plat. aggreg. EC_{50} [μM]
4	ridogrel		$C_{18}H_{17}F_3N_2O_3$		1.7[c]	0.004[d]	16
5	samixogrel		$C_{25}H_{25}ClN_2O_4S$	164	0.019	0.004	1
26	NH_2	3	$C_{19}H_{19}N_5O_2$	174−175	0.035	0.43	1.0
27	CH_3−NH	3	$C_{20}H_{21}N_5O_2$	120−123	0.016	0.25	0.46
28	$(CH_3)_2N$	3	$C_{21}H_{23}N_5O_2$	194	0.12	0.026	1.0
29	$(CH_3)_2CH$−NH	3	$C_{22}H_{25}N_5O_2$	186	0.018	0.024	0.4
30	$(CH_3)_2CH-CH_2$−NH	3	$C_{23}H_{27}N_5O_2$	129[b]	0.007	0.025	0.35
31	$(CH_3)_2CH-CH_2-CH_2$−NH	3	$C_{24}H_{29}N_5O_2$	108[b]	0.027	0.003	0.69
32	$(CH_3)_3C$−NH	3	$C_{23}H_{27}N_5O_2$	147[b]	0.011	0.004	0.31
33	cyclopropylamino	3	$C_{22}H_{23}N_5O_2$	178[b]	0.017	0.38	0.9
34	cyclopropylamino	4	$C_{22}H_{23}N_5O_2$	118−119	21	3.9	42
35	cyclopentylamino	3	$C_{24}H_{27}N_5O_2$	136−137[b]	0.03	0.003	0.5
36	cyclohexylamino	3	$C_{25}H_{29}N_5O_2$	156−157[b]	0.047	0.003	5.8
37	cyclohexylamino	4	$C_{25}H_{29}N_5O_2$	145−147	1.3	0.18	31
38	1-adamantylamino	3	$C_{29}H_{33}N_5O_2$	198	0.083	0.003	0.5
39	$C_6H_5-CH_2$−NH	3	$C_{26}H_{25}N_5O_2$	138−140	0.032	0.011	1.6
40	3-pyridyl−CH_2−NH	3	$C_{25}H_{26}N_6O_2{\cdot}0.5H_2O$	125−126	0.13	0.32	6.9
41	$(CH_3)_2N-CH_2-CH_2$−NH	3	$C_{23}H_{28}N_6O_2{\cdot}H_2O$	foam	1	2.9	6.8

[a] For details, see Experimental Section. [b] Decomposition. [c] 5.2 μM (ref 14b). [d] 0.008 μM (ref 14c).

cell membrane penetration. This hypothesis is supported by the observation that the introduction of hydrophilic groups (**40, 41**) decreases the synthase inhibitory activity. It is of interest that the size and

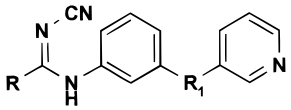
Table 2. Physical Constants and in Vitro Activity of Compounds[a]



no.	R	A	formula	mp [°C]	TXA_2 receptor antagonis IC_{50} [μM]	TXA_2 synthase inhibition IC_{50} [μM]	Inh. of collagen-induced plat. aggreg. EC_{50} [μM]
42	$(CH_3)_3C-NH$	C=O	$C_{22}H_{27}N_3O_3$	165	1.3	0.04	9
43	$(CH_3)_3C-NH$	C=S	$C_{22}H_{27}N_3O_2S$	137−139[b]	0.038	0.022	2
44	cyclopentylamino	C=NH	$C_{23}H_{28}N_4O_2 \cdot 0.5H_2O$	150	1.7	3.9	21
45	$(CH_3)_2CH-CH_2-NH$	$=N-CONH_2$	$C_{23}H_{29}N_5O_3$	foam	0.61	0.2	12
46	cyclopentylamino	=N−COPh	$C_{30}H_{32}N_4O_3$	171	0.11	0.004	16
47	$(CH_3)_3C-NH$	$=N-SO_2CH_3$	$C_{23}H_{30}N_4O_4S$	150−151	0.33	0.025	6.1
48	$(CH_3)_3C-NH$	$=N-SO_2Ph$	$C_{28}H_{32}N_4O_4S$	foam	0.055	0.003	3.3
49	$(CH_3)_2CH-CH_2-NH$	=N-5-tetrazolyl	$C_{23}H_{28}N_8O_2$	218[b]	0.3	0.93	22
50	cyclohexylamino	$=CH-NO_2$	$C_{25}H_{30}N_4O_4$	155−157	0.44	0.31	15
51	$(CH_3)_3C-NH$	$C=C(CN)_2$	$C_{25}H_{27}N_5O_2$	188−189	0.003	0.05	0.12
52	$(CH_3)_3C-NH$		$C_{25}H_{27}N_3O_4$	150[b]	0.83	0.23	59

[a] For details, see Experimental Section. [b] Decomposition.

Table 3. Physical Constants and in Vitro Activity of Compounds[a]



no.	R	R1	config	formula	mp [°C]	TXA_2 receptor antagonism IC_{50} [μM]	TXA_2 synthetase inhibition IC_{50} [μM]	Inh. of collagen-induced plat. aggreg. EC_{50} [μM]
53	$(CH_3)_3C-NH$	$C=CH-(CH)_3-COOH$	*Z*	$C_{23}H_{27}N_5O_2$	162	2.6	1.6	48
58	$(CH_3)_3C-NH$	$C=CH-(CH)_2-COOH$	*E*	$C_{22}H_{25}N_5O_2$	146−148	0.081	0.4	13
59	$(CH_3)_3C-NH$	$C=CH-(CH)_4-COOH$	*E*	$C_{24}H_{29}N_5O_2$	96	0.33	0.006	3.7
60	$(CH_3)_3C-NH$	$C=CH-(CH)_5-COOH$	*E*	$C_{25}H_{31}N_5O_2$	136−138	0.077	0.005	1.7
64	$(CH_3)_3C-NH$	$=N-O-(CH_2)_4-COOH$	*E*	$C_{23}H_{28}N_6O_3$	161−162	0.31	0.005	5.2
65	$(CH_3)_3C-NH$	$=N-O-(CH_2)_4-COOH$	*Z*	$C_{23}H_{28}N_6O_3$	172−173	0.51	0.23	17

[a] For details, see Experimental Section.

shape of the alkyl group have only a minor effect on the receptor antagonism too.

The importance of the meta position of the *N*-cyanoguanidine group in contrast to the structure−activity relationships (SARs) of **5** is demonstrated by the isomers **33**/**34** and **36**/**37**. Receptor antagonistic as well as synthase inhibitory activities are significantly reduced by a change from the meta to the para position.

To get deeper insight into the SARs we have varied the *N*-cyano part at the guanidine group. Replacement of the *N*-cyano group simply by oxygen (**42**) or nitrogen (**44**) reduces both TxRA and TxSI. This effect is not so pronounced if the *N*-cyano group is replaced by sulfur (**43**). Replacement of the cyano group by a carbamoyl group (**45**) is associated with a significant decrease in receptor antagonism and synthase inhibition whereas the benzoyl derivative **46** reveals only a reduced TxRA activity. We suppose that the increased lipophilicity of compound **46** compared to **45** is responsible for the improved synthase inhibition.

Comparable structure−activity relationships are found for the corresponding methylsulfonyl **47** and phenylsulfony **48** derivatives. Substitution of the *N*-cyano group by a tetrazole (**49**) or a nitro-methylene group (**50**) reduces both TxSI and TxRA activities.

Replacement of the *N*-cyano part by a dicyanomethylene group (**51**) results in the most potent TXA_2 receptor antagonist we have found within our research program. Unfortunately the TxSI is reduced by a factor of 10 compared to the *N*-cyano guanidine **32**. Both activities are reduced if the guanidine moiety is substituted by a cyclobutenedione group (**52**).

Further investigations concentrated on the influence of the carboxyalkyl chain. It is not surprising that SARs for this part of compound **32** are very similar to those found for compound **5**. The importance of the *E*-configuration is obvious by comparison of the isomers **32** and **53**. The butenoic acid derivative **58** is a weak TxSI due to the fact that the distance between the pyridine nitrogen atom and the carboxyl acid group is not optimal. The derivatives **59** and **60** with prolonged carboxyalkenyl chains are potent synthase inhibitors, but TxRA activity is reduced compared to **32**.

Finally we have synthesized the *E*- and *Z*-isomers **64** and **65** which are direct analogues to ridogrel **4**. As we expected, the *E*-isomer **64** is as potent as ridogrel with

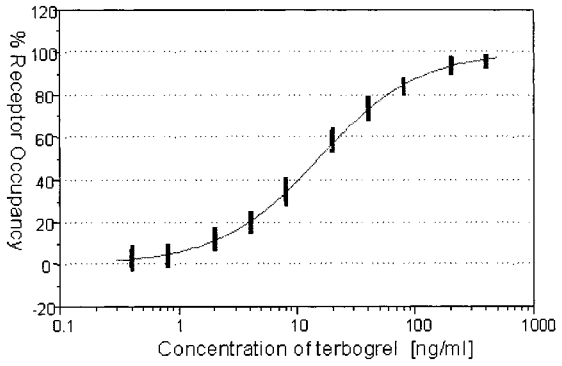


Figure 3. Summary of in vitro studies in which various amounts of terbogrel were added to platelet-rich plasma collected from 15 human volunteers and then the receptor occupancy was determined. In this system, the IC_{50} value was 30 nmol/L (note: in figure, concentration is given in ng/mL).

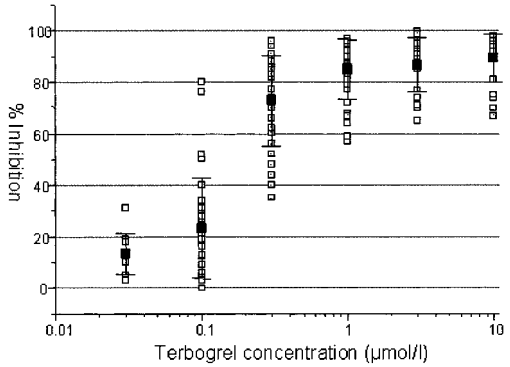


Figure 4. Summary of collagen-induced (1 μg/mL) aggregation in platelet-rich plasma in response to various concentrations of terbogrel. Individual data points ($N = 20$ in duplicate) as well as the mean and standard deviation are shown.

regard to synthase inhibition, but the receptor antagonism is significantly reduced in comparison to **32**. It is of interest that both isomers **64** and **65** are more potent as receptor antagonists compared to the parent compound **4**.

From the series of *N*-cyano guanidine derivatives described, compound **32** (INN: terbogrel) was selected for further investigations due to its favorable physicochemical properties as well as its strong and balanced receptor blockade and synthase inhibition. In a competitive binding assay with the selective TXA_2 receptor blocker SQ 29,448 in gel-filtered human platelets, terbogrel exhibited an IC_{50} of 11 nmol/L. Since binding to plasma proteins may cause a loss of efficacy in vivo, it is important to also test for receptor affinity in the presence of plasma proteins. In a test system using human platelet-rich plasma, as opposed to gel-filtered platelets, such that plasma proteins are present, terbogrel **32** demonstrated an IC_{50} of 38 ± 1 nmol/L (n = 15, Figure 3). Thus, there is little if any effect of plasma proteins on the affinity of terbogrel to the TXA_2 receptor in contrast to samixogrel **5** (IC_{50} = 790 nmol/L). This result may be due to the decreased lipophilicity of **32** (log P (*n*-octanol/buffer pH 7.4) = 1.04) and the increased solubility in aqueous buffer (5.4 mg/100 mL at pH 7) in comparison to **5** (log P = 2.36; solubility = 0.14 mg/100 mL at pH 7).

The inhibition of TXA_2 formation and binding leads to an inhibition of aggregation of collagen-stimulated platelets. In platelet-rich plasma, terbogrel **32** inhibited platelet aggregation stimulated by 1 μg/mL collagen with an IC_{50} of 310 ± 18 nmol/L (n = 8) (Figure 4). This experimental system for profiling agents such as terbogrel with a dual inhibition of thromboxane must be viewed with caution, however. In platelet-rich plasma, no cells capable of prostacyclin synthesis are present thereby preventing this additional antiaggregatory mechanism in this experimental system.

If the redirection of arachidonic acid metabolites to prostacyclin synthesis is of relevance, one would predict that terbogrel has a higher antiaggregatory potency in test systems in which prostacyclin synthesis can occur. In the studies with whole blood aggregation, terbogrel had an IC_{50} of 52 ± 20 nmol/L (n = 6) which indicates a substantially higher (~6-fold) antiaggregatory potency in this system as compared to platelet-rich plasma. In whole blood, in contrast to platelet-rich plasma, white blood cells are present which can convert prostaglandin endoperoxides to prostacyclin thereby accounting for the markedly higher potency of terbogrel in the whole blood system. This is further supported by the measurement of 6-keto-prostaglandin $F_{1\alpha}$ in plasma from these studies. With the addition of the collagen stimulus (1 μg/mL) there was a 6-fold increase in the concentration of 6-keto-prostaglandin $F_{1\alpha}$ in the plasma over that induced by collagen without terbogrel. It is tempting to attribute the higher antiaggregatory potency of terbogrel in whole blood, as compared to platelet-rich plasma, to the production of prostacyclin, as measured as its stable metabolite 6-keto-prostaglandin $F_{1\alpha}$. This gives good supporting evidence for the existence of the combined TXA_2 receptor blockade and synthase inhibition mechanism in this system. Between 10 and 30 μM terbogrel, the level of 6-keto-prostaglandin $F_{1\alpha}$ began to decrease which is probably due to cyclooxygenase inhibition at these high concentrations. Iteration of the biphasic dose−response curve resulted in an EC_{50} of 0.018 μM for 6-keto-prostaglandin $F_{1\alpha}$ increase and an IC_{50} of 50 μM for the presumed cyclooxygenase inhibition, respectively.

In Vivo Pharmacology

Acute damage of an artery exposes thrombogenic components of the vessel wall to the flowing blood, which causes platelet activation and thrombus formation. In the presence of a stenosis and the increased shear forces thereby produced, an occlusive arterial thrombus readily forms. Folts demonstrated that the mechanical removal of such platelet-rich thrombi in the coronary artery of an anesthetized dog could temporarily restore blood flow through the artery but that a new thrombus quickly forms to reocclude the vessel.[31] He exploited this phenomenon of recurrent coronary thrombosis to make a widely used model for the testing of antithrombotic agents.[32] Effective antithrombotic agents are those that can either slow or prevent the coronary thrombus formation.

Doses of 0.1−3.0 mg/kg were administered as an intravenous bolus in rabbits having recurrent arterial thrombosis. A dose-dependent effect was seen primarily in the length of thrombus inhibition since all doses produced similar effects immediately after bolus administration. A 50% inhibition throughout the 1 h observation period could be produced by a dose of 0.19

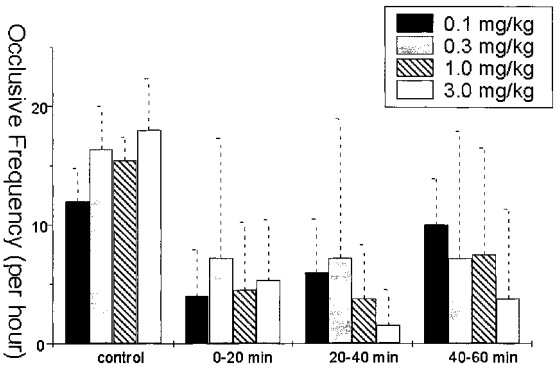


Figure 5. Summary of occlusive frequency (number of occlusive thrombi per hour) for the four terbogrel dose groups tested (mean + SD) in the hour following bolus intravenous administration.

± 0.07 mg/kg (n = 20) (Figure 5). When one considers the weaker TXA_2 receptor binding affinity to rabbit platelets in comparison to man (a 190-fold difference), terbogrel demonstrated an impressive antithrombotic efficacy.

In basic pharmacokinetic studies with radiolabeled terbogrel in rats (10 mg/kg po), the compound is rapidly and well (90%) absorbed with a systemic availability of about 30%. The drug is mainly excreted (>80%) in the bile, and the elimination occurred almost exclusively in the feces. The terminal half-life in rats after oral application is in the range from 7.5 to 10 h.

Conclusion

We succeeded in the synthesis of combined TXA_2 receptor antagonists and synthase inhibitors by replacement of the phenylsulfonamide moiety of samixogrel **5** by alkyl-substituted nonbasic guanidines. The most active compounds are *N*-cyano-guanidines that bind to the TXA2/endo receptor and inhibit the TXA_2 synthase in the lower nanomolar range. Due to its potent antiaggregatory activity in vitro as well as in vivo, terbogrel **32** was selected for clinical investigations.

Experimental Section

(a) Chemistry. Melting points were determined in a Büchi capillary melting point apparatus and were not corrected. Infrared (IR) spectra were recorded on a Perkin-Elmer model 298 spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AC200 and a Bruker AMX400 instrument. Chemical shifts are reported in δ units relative to internal tetramethylsilane. Mass spectra were recorded on a Finnigan MAT 8230 or an AEI MS-902 mass spectrometer in either EI or fast-atom-bombardment mode. Microanalyses were performed on a CHN-Rapid (Heraeus). Silica gel (Baker 30−60 μm) was used for column chromatography. TLC was performed on silica gel plates (Macherey-Nagel Polygram Sil G/UV 254 or Merck, silica gel 60, F-254).

Methyl 5*E*-6-(3-(Cyanimido-phenoxymethylenamino)-phenyl)-6-(3-pyridyl)hex-5-enoate (10). Compounds **8** (10.5 g, 35 mmol) and **9** (8.4 g, 35 mmol) were dissolved in ^{i}PrOH (100 mL) and stirred for 5 h at 20 °C. The reaction mixture was concentrated, and the residue was chromatographed on silica gel with CH_2Cl_2/EtOH 40:1 to yield 13.3 g (86%) of **10** as an oil: ^{1}H NMR ($CDCl_3$) 1.8 (q, 2H), 2.3 (m, 4H), 3.6 (s, 3H), 6.1 (t, 1H), 6.8−7.5 (m, 15H), 8.5 (d + dd, 2H), 8.8 (s, 1H); IR (CH_2Cl_2) 2160 (CN), 1730 (C=O) cm^{-1}. Anal. ($C_{26}H_{24}N_4O_3$) C, H, N.

5*E*-6-(4-Acetamido-phenyl)-6-(3-pyridyl)hex-5-enoic acid (12). Compound **11** (140 g, 580 mmol) was added to a mixture of 4-carboxybutyl-triphenylphosphonium bromide (307.2 g, 690 mmol) and KOtBu (232.9 g, 2080 mmol) in THF (2800 mL) at −30 °C. After 2 h of being stirred at 20 °C, the reaction mixture was combined with water (500 mL) and then evaporated. The residue was taken up in water (1000 mL) and washed with EtOAc. The aqueous phase was neutralized by addition of citric acid and extracted with EtOAc. The organic phase was evaporated, and the residue was then recystallized from EtOAc/diisopropyl ether and dried at 80 °C to yield 154 g (86%) of **12**, mp 155−156 °C: ^{1}H NMR (DMSO-d_6) 1.7 (q, 2H), 2.05 (s, 3H), 2.2 (m, 4H), 6.2 (t, 1H), 6.85 (d, 1H), 7.35 (m, 3H), 7.6 (m, 2H), 8.4 (m, 2H), 9.9 (s, 1H), 12.0 (bs, 1H); Anal. ($C_{19}H_{20}N_2O_3$) C, H, N.

Methyl 5*E*-6-(4-(Cyanimido-phenoxymethylenamino)-phenyl)-6-(3-pyridyl)hex-5-enoate (13). Compound **12** (16.2 g, 50 mmol) was stirred in MeOH (70 mL) and 7 M HCl in MeOH (100 mL) for 12 h at 20 °C. Water (200 mL) was added, and the reaction mixture was adjusted to pH 9 by addition of ammonia and immediately extracted with EtOAc. The organic phase was washed with water, dried, and concentrated. The residue was dissolved in ^{i}PrOH (250 mL), and **9** (11.4 g, 50 mmol) was added. The mixture was stirred for 6 h at 25 °C, the crystals formed were filtered with suction and washed with Et_2O to yield 19.1 g (86%) of **15**, mp 163−165 °C: ^{1}H NMR (DMSO-d_6) 1.7 (q, 2H), 2.1 (q, 2H), 2.3 (t, 2H), 3.5 (s, 3H), 6.2 (t, 1H), 7.15 (d, 2H), 7.25−7.6 (m, 11H), 8.45 (m, 2H), 10.9 (s, 1H); IR (CH_2Cl_2) 2200 (CN), 1730 (C=O) cm^{-1}. Anal. ($C_{26}H_{24}N_4O_3$) C, H, N.

Methyl 5*E*-6-(3-(Benzoylimido-phenoxymethylenamino)phenyl)-6-(3-pyridyl)hex-5-enoate (15). Compounds **8** (4.14 g, 14 mmol) and **14** (5 g, 35 mmol) were dissolved in ^{i}PrOH (200 mL) and stirred for 4 h at 20 °C. The reaction mixture was chilled to 0 °C, and the crystals formed were filtered with suction to yield 5.8 g (80%) of **15**, mp 112 °C: ^{1}H NMR ($CDCl_3$) 1.7 (q, 2H), 2.25 (m, 4H), 3.6 (s, 3H), 6.1 (t, 1H), 7.0 (d, 1H), 7.1−7.5 (m, 13H), 7.95 (d, 2H), 8.5 (d + dd, 2H); IR (CH_2Cl_2) 1730 (C=O) cm^{-1}. Anal. ($C_{32}H_{29}N_3O_4$) C, H, N.

Methyl 5*E*-6-(3-(1-Methylthio-2-nitro-ethylenamino)-phenyl)-6-(3-pyridyl)hex-5-enoate (17). Compounds **8** (3 g, 10 mmol) and **16** (1.65 g, 10 mmol) were refluxed in ^{i}PrOH (50 mL) for 20 h. The mixture was filtered and evaporated, and the residue was purified over a silica gel column using CH_2Cl_2/EtOH 30:1 and subsequently recrystallized from TBME/diisopropyl ether to yield 2.6 g (63%) of **17**, mp 84 °C: ^{1}H NMR ($CDCl_3$) 1.8 (q, 2H), 2.2 (t, 4H), 2.3 (q, 2H), 2.4 (s, 3H), 3.65 (s, 3H), 6.15 (t, 1H), 6.7 (s, 1H), 7.1−7.3 (m, 4H), 7.45 (m, 2H), 8.5 (m, 2H), 11.8 (s, 1H); IR (CH_2Cl_2) 1735 (C=O) cm^{-1}. Anal. ($C_{21}H_{23}N_3O_4S$) C, H, N, S.

***N*-Diphenoxymethylenephenylsulfonamide (19).** Dichloro-diphenoxymethane (13.5 g, 50 mmol) and benzenesulfonamide (17.3 g, 110 mmol) were refluxed in EtOAc (60 mL) for 55 h. The reaction mixture was washed with sodium bicarbonate solution and water. The organic phase was dried, concentrated and the residue was extracted with hot CH_2Cl_2. The dichloromethane solution was evaporated and the residue was purified by column chromatography using CH_2Cl_2 followed by recrystallization from EtOAc/diisopropyl ether to yield 4.6 g (26%) of **19**, mp 121−122 °C: ^{1}H NMR ($CDCl_3$) 7.05 (m, 4H), 7.2−7.6 (m, 9H), 7.95 (dd, 2H); Anal. ($C_{19}H_{15}NO_4S$) C, H, N, S.

Methyl 5*E*-6-(3-(methylsulfonimido-phenoxymethylenamino)phenyl)-6-(3-pyridyl)-hex-5-enoate (20). Compounds **8** (7.4 g, 25 mmol) and **18** (7.3 g, 25 mmol) were stirred in ^{i}PrOH (250 mL) for 24 h at 25 °C. The mixture was concentrated, and the residue was purified by column chromatography with CH_2Cl_2/EtOH 20:1 followed by recrystallization from EtOAc/petroleum ether to yield 10.4 g (84%) of **20**, mp 150−151 °C: ^{1}H NMR ($CDCl_3$) 1.75 (q, 2H), 2.2 (m, 4H), 2.9 (s, 3H), 3.6 (s, 3H), 6.1 (t, 1H), 7.0−7.5 (m, 11H), 8.5 (d + dd,2H), 9.25 (s, 1H); IR (CH_2Cl_2) 1740 (C=O) cm^{-1}. Anal. ($C_{26}H_{27}N_3O_5S$) C, H, N, S.

Methyl 5*E*-6-(3-(phenylsulfonimido-phenoxymethylenamino)phenyl)-6-(3-pyridyl)-hex-5-enoate (21). Compounds **8** (3 g, 10 mmol) and **19** (3.5 g, 10 mmol) were stirred in ^{i}PrOH (60 mL) for 48 h at 25 °C. The mixture was

concentrated, and the residue was purified by column chromatography with CH_2Cl_2 and by recrystallization from diethyl ether/petroleum ether to yield 4.8 g (86%) of **21**, mp 90−92 °C: ^{1}H NMR ($CDCl_3$) 1.75 (q, 2H), 2.2 (m, 4H), 3.6 (s, 3H), 6.1 (t, 1H), 7.1 (m, 3H), 7.1−7.55 (m, 11H), 7.8 (m, 2H), 8.5 (m, 2H), 9.5 (s, 1H); IR (CH_2Cl_2) 1740 (C=O) cm^{-1}. Anal. ($C_{31}H_{29}N_3O_5S$) C, H, N, S.

Methyl 5*E*-6-(3-(2,2-Dicyano-1-methylthio-ethylenamino)phenyl)-6-(3-pyridyl)hex-5-enoate (23). Compounds **8** (13.4 g, 45 mmol) and **22** (7.7 g, 45 mmol) were refluxed in ^{i}PrOH (130 mL) for 6−8 h. The reaction mixture was evaporated, and the residue was recrystallized from EtOAc/diisopropyl ether to yield 8.4 g (44%) of **23**, mp 125−127 °C: ^{1}H NMR ($CDCl_3$) 1.8 (q, 2H), 2.2 (q, 2H), 2.35 (t + s, 5H), 3.65 (s, 3H), 6.15 (t, 1H), 7.05 (d, 1H), 7.1−7.3 (m, 3H), 7.4−7.6 (m, 2H), 8.45 (d + dd, 2H), 8.6 (bs, 1H); IR (CH_2Cl_2) 2220 (CN), 1740 (C=O) cm^{-1}. Anal. ($C_{23}H_{22}N_4O_2S$) C, H, N, S.

Methyl 5*E*-6-(3-(2,3-dioxo-4-ethoxy-cyclobut-4-enyl)-aminophenyl)-6-(3-pyridyl)-hex-5-enoate (25). Compounds **8** (3 g, 10 mmol) and **24** (1.7 g, 10 mmol) in ^{i}PrOH (50 mL) were stirred for 20 h at 25 °C. The reaction mixture was concentrated, and the residue was purified by column chromatography with CH_2Cl_2/EtOH 20:1 to yield 3.3 g (78%) of **25** as a viscous oil: ^{1}H NMR ($CDCl_3$) 1.4 (t, 3H), 1.8 (q, 2H), 2.15−2.4 (m, 4H), 3.65 (s, 3H), 4.8 (q, 2H), 6.1 (t, 1H), 6.9 (m, 1H), 7.1−7.4 (m, 4H), 7.5 (dt, 1H), 8.2 (bs, 1H), 8.5 (m, 2H); IR (CH_2Cl_2) 1810, 1745 (C=O) cm^{-1}. Anal. ($C_{24}H_{24}N_2O_5$) C, H, N.

5*E*-6-(3-Cyanoguanidino)phenyl-6-(3-pyridyl)hex-5-enoic Acid (26). Compound **10** (2.2 g, 5 mmol) and $(NH_4)_2CO_3$ (4.8 g, 50 mmol) were dissolved in MeOH (40 mL) and stirred at 20 °C for 48 h. The reaction mixture was concentrated, and the residue was dissolved in water. The aqueous phase was extracted, with EtOAc. The organic phase was evaporated and the residue was dissolved in ^{i}PrOH (20 mL) and 5M NaOH (5 mL). The solution was heated to 50 °C for 0.5 h and concentrated, and the residue was taken up in water. The aqueous phase was extracted with EtOAc and acidified by addition of citric acid, and the precipitate was filtered with suction. Purification was effected by column chromatography on silica gel using CH_2Cl_2/EtOH 9:1 followed by recrystallization from ^{i}PrOH/EtOAc; yield 0.68 g (38%): ^{1}H NMR (DMSO-d_6) 1.7 (q, 2H), 2.2 (m, 4H), 6.2 (t, 1H), 6.85 (m, 1H), 7.0 (s, 2H), 7.1 (s, 1H), 7.35 (m, 3H), 7.55 (dt, 1H), 8.45 (m, 2H), 9.1 (s, 1H), 12.0 (s, 1H); IR (KBr) 2200 (CN) cm^{-1}. Anal. ($C_{19}H_{19}N_5O_2$) C, H, N.

5*E*-6-(3-(2-Cyano-3-methyl)guanidino)phenyl)-6-(3-pyridyl)hex-5-enoic acid (27). Compound **10** (2.2 g, 5 mmol) and methylamine (2 mL, 50 mmol) in ^{i}PrOH (20 mL) were heated in a sealed tube to 50 °C for 5 h. The workup was performed according to **26**. Purification on silica gel with CH_2Cl_2/EtOH 15:1 and subsequent recrystallization from EtOAc/IPrOH yielded 0.9 g (49%) of **27**: ^{1}H NMR (DMSO-d_6) 1.7 (q, 2H), 2.2 (m, 4H), 2.8 (d, 3H) 6.2 (t, 1H), 6.9 (dd, 1H), 7.05 (s, 1H), 7.2−7.4 (m, 3H), 7.6 (dt, 1H), 8.45 (m, 2H), 8.9 (s, 1H), 12.0 (s, 1H); IR (KBr) 2180 (CN) cm^{-1}. Anal. ($C_{20}H_{21}N_5O_2$) C, H, N.

5*E*-6-(3-(2-Cyano-3,3-dimethyl)guanidino)phenyl)-6-(3-pyridyl)hex-5-enoic acid (28). Preparation was performed according to **27** starting from **10** (1.45 g, 3.3 mmol) and dimethylamine (3 g, 70 mmol). Purification on silica gel with CH_2Cl_2/EtOH 9:1 and subsequent recrystallization from EtOAc/diisopropyl ether yielded 0.3 g (24%) of **28**: ^{1}H NMR (DMSO-d_6) 1.7 (q, 2H), 2.2 (m, 4H), 3.0 (s, 6H), 6.2 (t, 1H), 6.8 (m, 2H), 7.05 (s, 1H), 7.2−7.4 (m, 2H), 7.6 (dt, 1H), 8.45 (m, 2H), 9.15 (s, 1H), 12.0 (s, 1H); IR (KBr) 2160 (CN) cm^{-1}. Anal. ($C_{21}H_{23}N_5O_2$) C, H, N.

5*E*-6-(3-(2-Cyano-3-isopropyl)guanidino)phenyl)-6-(3-pyridyl)hex-5-enoic acid (29). Compound **10** (1.85 g, 4.2 mmol) and isopropylamine (2 mL, 23 mmol) in ^{i}PrOH (30 mL) were refluxed for 0.5 h. The reaction mixture was chilled to 25 °C, 2 M NaOH (4 mL) was added, and the solution was stirred at 25 °C for 4 h. The mixture was concentrated, and the residue was taken up in water (50 mL) and acidified by addition of citric acid. The precipitate was filtered with suction and recrystallized from EtOH/EtOAc to yield 0.83 g (50%) of **29**: ^{1}H NMR (DMSO-d_6) 1.1 (d, 6H), 1.7 (q, 2H), 2.2 (m, 4H), 4.0 (sep, 1H), 6.2 (t, 1H), 6.9 (d, 1H), 7.0 (s, 1H), 7.1 (d, 1H), 7.2−7.4 (m, 3H), 7.6 (dd, 1H), 8.45 (m, 2H), 9.0 (s, 1H), 12.0 (s, 1H); IR (KBr) 2160 (CN) cm^{-1}. Anal. ($C_{22}H_{25}N_5O_2$) C, H, N.

5*E*-6-(3-(2-Cyano-3-(2-methylpropyl)guanidino)phenyl)-6-(3-pyridyl)hex-5-enoic Acid (30). Preparation was performed according to **29**. Purification was effected by column chromatography with 1,2-dichloroethane/EtOH 20:1 and by subsequent recrystallization from EtOAc/diisopropyl ether; yield 74%: ^{1}H NMR (DMSO-d_6) 0.85 (d, 6H), 1.7 (q, 2H), 1.8 (sep, 1H), 2.2 (m, 4H), 3.5 (t, 2H), 6.2 (t, 1H), 6.9 (d, 1H), 7.0 (s, 1H), 7.2−7.45 (m, 4H), 7.6 (dd, 1H), 8.45 (m, 2H), 9.0 (s, 1H), 12.0 (s, 1H); IR (KBr) 2170 (CN) cm^{-1}. Anal. ($C_{23}H_{27}N_5O_2$) C, H, N.

5*E*-6-(3-(2-Cyano-3-(3-methylbutyl)guanidino)phenyl)-6-(3-pyridyl)hex-5-enoic Acid (31). Preparation was performed according to **29**. Purification was effected by column chromatography with CH_2Cl_2/EtOH 15:1 and by subsequent recrystallization from EtOAc/TBME; yield 71%: ^{1}H NMR ($CDCl_3$) 0.9 (d, 6H), 1.45 (q, 2H), 1.6 (sep, 1H), 1.85 (m, 2H), 2.25 (m, 2H), 2.4 (m, 2H), 3.35 (m, 2H), 5.45 (t, 1H), 6.1 (t, 1H), 6.9 (d, 1H), 7.0 (s, 1H), 7.15−7.45 (m, 4H), 7.6 (dd, 1H), 8.45 (s, 1H), 8.5 (m, 2H); IR (KBr) 2170 (CN) cm^{-1}. Anal. ($C_{24}H_{29}N_5O_2$) C, H, N.

5*E*-6-(3-(2-Cyano-3-*tert*-butyl-guanidino)phenyl)-6-(3-pyridyl)hex-5-enoic acid (32) and 5*Z*-6-(3-(2-Cyano-3-*tert*-butyl-guanidino)phenyl)-6-(3-pyridyl)hex-5-enoic acid (53). Preparation was performed according to **29**. Purification was effected by recrystallization from EtOAc/diisopropyl ether; yield 45%: ^{1}H NMR (DMSO-d_6) 1.3 (s, 9H), 1.65 (q, 2H), 2.2 (m, 4H), 6.2 (t, 1H), 6.85 (m, 3H), 7.1 (d, 1H), 7.25−7.4 (dd + t, 2H), 7.6 (dt, 1H), 8.45 (m, 2H), 9.0 (s, 1H), 12.0 (s, 1H); IR (KBr) 2160 (CN) cm^{-1}. Anal. ($C_{23}H_{27}N_5O_2$) C, H, N.

The mother liquid was concentrated and the residue was twice chromatographed on silica gel with EtOAc/CH_2Cl_2/acetic acid 55:45:5. The faster running fractions were collected and evaporated, the residue was taken up in a minimum of EtOAc, and the *Z*-isomer **53** was precipitated by addition of diethyl ether; yield 3%: ^{1}H NMR (DMSO-d_6) 1.25 (s, 9H), 1.65 (q, 2H), 2.05 (q, 2H), 2.2 (t, 2H), 3.2 (m, 2H), 6.2 (t, 1H), 6.65 (s, 1H), 6.85 (s, 1H), 7.0 (t, 2H), 7.25 (t, 1H), 7.45 (dd, 1H), 7.6 (dt, 1H), 8.35 (d, 1H), 8.55 (dd, 1H), 8.9 (s, 1H), 12.0 (s, 1H); IR (KBr) 2170 (CN) cm^{-1}. Anal. ($C_{23}H_{27}N_5O_2$) C, H, N.

5*E*-6-(3-(2-Cyano-3-cyclopropyl-guanidino)phenyl)-6-(3-pyridyl)hex-5-enoic Acid (33). Preparation was performed according to **29**. Purification was effected by recrystallization from water/^{i}PrOH; yield 46%: ^{1}H NMR (DMSO-d_6) 0.6 (m, 2H), 0.75 (m, 2H), 1.7 (q, 2H), 2.2 (m, 4H), 2.65 (m, 1H), 6.2 (t, 1H), 6.9 (m, 1H), 7.1 (d, 1H), 7.25−7.4 (m, 3H), 7.6 (dd, 1H), 7.65 (s, 1H), 8.45 (m, 2H), 8.85 (s, 1H), 12.0 (s, 1H); IR (KBr) 2170 (CN) cm^{-1}. Anal. ($C_{22}H_{23}N_5O_2$) C, H, N.

5*E*-6-(4-(2-Cyano-3-cyclopropyl-guanidino)phenyl)-6-(3-pyridyl)hex-5-enoic Acid (34). Preparation was performed according to **29** starting from **13**. Purification was effected by recrystallization from EtOAc/^{i}PrOH; yield 28%: ^{1}H NMR (DMSO-d_6) 0.65 (m, 2H), 0.8 (m, 2H), 1.7 (q, 2H), 2.2 (m, 4H), 2.7 (m, 1H), 6.2 (t, 1H), 7.1 (d,2H), 7.25−7.45 (m, 3H), 7.55 (dd, 1H), 7.8 (d, 1H), 8.45 (m, 2H), 8.9 (s, 1H), 12.0 (s, 1H); IR (KBr) 2170 (CN) cm^{-1}. Anal. ($C_{22}H_{23}N_5O_2$) C, H, N.

5*E*-6-(3-(2-Cyano-3-cyclopentyl-guanidino)phenyl)-6-(3-pyridyl)hex-5-enoic Acid (35). Preparation was performed according to **29**. Purification was effected by recrystallization from EtOAc/diethyl ether; yield 66%: ^{1}H NMR (DMSO-d_6) 1.4−2.0 (m, 10H), 2.2 (m, 4H), 4.1 (m, 1H), 6.2 (t, 1H), 6.9 (d, 1H), 7.0 (s, 1H), 7.2−7.4 (m, 4H), 7.6 (dd, 1H), 8.45 (m, 2H), 8.95 (s, 1H), 12.0 (s, 1H); IR (KBr) 2180 (CN) cm^{-1}. Anal. ($C_{24}H_{27}N_5O_2$) C, H, N.

5*E*-6-(3-(2-Cyano-3-cyclohexyl-guanidino)phenyl)-6-(3-pyridyl)hex-5-enoic Acid (36). Preparation was performed according to **29**. Purification was effected by recrystallization from EtOAc/diethyl ether; yield 62%: ^{1}H NMR (DMSO-d_6) 1.1−1.4 (m, 6H), 1.5−1.9 (m, 7H), 2.2 (m, 4H), 3.6 (m, 1H), 6.2 (t, 1H), 6.9 (d, 1H), 7.0 (s, 1H), 7.1 (d, 1H), 7.2−7.4 (m,

3H), 7.6 (dd, 1H), 8.45 (m, 2H), 9.0 (s, 1H), 12.0 (s, 1H); IR (KBr) 2180 (CN) cm^{-1}. Anal. ($C_{25}H_{29}N_5O_2$) C, H, N.

5*E*-6-(4-(2-Cyano-3-cyclohexyl-guanidino)phenyl)-6-(3-pyridyl)hex-5-enoic Acid (37). Preparation was performed according to **29** starting from **13**. Purification was effected by recrystallization from EtOAc/^{i}PrOH; yield 39%: ^{1}H NMR (DMSO-d_6) 1.1−1.4 (m, 5H), 1.5−1.95 (m, 7H), 2.2 (m, 4H), 3.65 (m, 1H), 6.2 (t, 1H), 7.05−7.2 (m, 3H), 7.25−7.4 (m, 3H), 7.6 (dd, 1H), 8.4 (m, 2H), 9.0 (s, 1H), 12.0 (s, 1H); IR (KBr) 2160 (CN) cm^{-1}. Anal. ($C_{25}H_{29}N_5O_2$) C, H, N.

5*E*-6-(3-(2-Cyano-3-(1-adamantyl)guanidino)phenyl)-6-(3-pyridyl)hex-5-enoic Acid (38). Preparation was performed according to **29**. Purification was effected by recrystallization from ^{i}PrOH/water; yield 64%: ^{1}H NMR (DMSO-d_6) 1.5−1.75 (m, 8H), 1.85−2.1 (m, 9H), 2.2 (m, 4H), 6.2 (t, 1H), 6.7 (s, 1H), 6.85 (m, 2H), 7.1 (d, 1H), 7.25−7.4 (m, 2H), 7.6 (dd, 1H), 8.45 (m, 2H), 9.05 (s, 1H), 12.0 (s, 1H); IR (KBr) 2180 (CN) cm^{-1}. Anal. ($C_{29}H_{33}N_5O_2$) C, H, N.

5*E*-6-(3-(2-Cyano-3-benzylguanidino)phenyl)-6-(3-pyridyl)hex-5-enoic Acid (39). Preparation was performed according to **29**. Purification was effected by column chromatography with CH_2Cl_2/EtOH 15:1 and by subsequent recrystallization from EtOAc/TBME; yield 53%: ^{1}H NMR (DMSO-d_6) 1.65 (q, 2H), 2.2 (m, 4H), 4.4 (d, 2H), 6.2 (t, 1H), 6.9 (d, 1H), 7.0 (d, 1H), 7.2−7.45 (m, 8H), 7.6 (dd, 1H), 7.8 (t, 1H), 8.45 (m, 2H), 9.15 (s, 1H), 12.0 (s, 1H); IR (KBr) 2160 (CN) cm^{-1}. Anal. ($C_{26}H_{25}N_5O_2$) C, H, N.

5*E*-6-(3-(2-Cyano-3-(3-pyridylmethyl)guanidino)phenyl)-6-(3-pyridyl)hex-5-enoic Acid (40). Preparation was performed according to **29**. Purification was effected by recrystallization from EtOAc/diisopropyl ether; yield 73%: ^{1}H NMR (DMSO-d_6) 1.65 (q, 2H), 2.2 (m, 4H), 4.4 (d, 2H), 6.2 (t, 1H), 6.95 (m, 2H), 7.2−7.45 (m, 4H), 7.55−7.85 (m, 3H), 8.45 (m, 4H), 9.25 (s, 1H), 12.0 (s, 1H); IR (KBr) 2190 (CN) cm^{-1}. Anal. ($C_{25}H_{24}N_6O_2 \cdot 0.5H_2O$) C, H, N.

5*E*-6-(3-(2-Cyano-3-(2-dimethylaminoethyl)guanidino)-phenyl)-6-(3-pyridyl)hex-5-enoic Acid (41). Preparation was performed according to **29**. Purification was effected by column chromatography with CH_2Cl_2/MeOH 9:1; yield 73%: ^{1}H NMR (DMSO-d_6) 1.65 (q, 2H), 2.1−2.3 (s + m, 10 H), 2.5 (m, 2H + DMSO), 3.35 (q, 2H), 6.2 (t, 1H), 6.9 (d, 1H), 7.1 (s, 1H), 7.2−7.4 (m, 4H), 7.6 (dt, 1H), 8.45 (m, 2H), 10.1 (s, 1H); IR (KBr) 2180 (CN) cm^{-1}. Anal. ($C_{23}H_{28}N_6O_2 \cdot H_2O$) C, H, N.

5*E*-6-3-(*tert*-Butylamino-carbonylamino)phenyl)-6-(3-pyridyl)hex-5-enoic Acid (42). Compound **8** (2.4 g, 8 mmol), *tert*-butylisocyanate (1 g, 10 mmol) and DMAP (50 mg) were refluxed in a mixture of THF (30 mL) and DMF (3 mL) for 12 h. The reaction mixture was poured on water (50 mL) and then extracted with EtOAc. The organic phase was concentrated, and the residue was stirred in EtOH (20 mL) and 2 N NaOH (7 mL) for 2 h at 40 °C. The solution was acidified with citric acid, and the precipitate was filtered with suction. Purification was performed by column chromatography on silica gel with CH_2Cl_2/EtOH 15:1 followed by recrystallization from EtOAc/diisopropyl ether; yield 1.6 g (52%): ^{1}H NMR (DMSO-d_6) 1.25 (s, 9H), 1.65 (q, 2H), 2.05−2.4 (m, 4H), 5.9 (s, 1H), 6.2 (t, 1H), 6.7 (m, 1H), 7.15 (s, 1H), 7.25−7.4 (m, 3H), 7.55 (dt, 1H), 8.3 (s, 1H), 8.45 (m, 2H), 12.0 (s, 1H); IR (KBr) 1695 (C=O) cm^{-1}. Anal. ($C_{22}H_{27}N_3O_3$) C, H, N.

5*E*-6-3-(*tert*-Butylamino-thiocarbonylamino)phenyl)-6-(3-pyridyl)hex-5-enoic Acid (43). Preparation was performed according to **42** starting from **8** (2.4 g, 8 mmol) and *tert*-butylisothiocyanate (1.15 g, 10 mmol). The reaction time was prolonged to 44 h. Recrystallization from EtOAc/TBME yields 0.33 g (10%): ^{1}H NMR (DMSO-d_6) 1.45 (s, 9H), 1.65 (q, 2H), 2.05−2.3 (m, 4H), 6.2 (t, 1H), 6.85 (d, 1H), 7.25−7.5 (m, 4H), 7.55 (dt, 1H), 8.45 (m, 2H), 9.35 (s, 1H). Anal. ($C_{22}H_{27}N_3O_2S$) C, H, N, S.

5*E*-6-(3-(3-Cyclopentylguanidino)phenyl)-6-(3-pyridyl-)hex-5-enoic Acid (44). Compound **46 (**1 g,2 mmol) was heated in 2 M HCl (20 mL) for 48 h at 95 °C. The reaction mixture was first neutralized with NH_3, and then the pH value was adjusted to 5 by addition of citric acid. The crystals formed were filtered with suction to yield 0.5 g (63%) of **44**: ^{1}H NMR (DMSO-d_6 + CD_3OD) 1.4−1.75 (m, 8H), 1.75−2.2 (m, 6H), 4.1 (m, 1H), 6.2 (t, 1H), 6.9 (d, 1H), 7.0 (s, 1H), 7.1 (d, 1H), 7.25−7.4 (m, 2H), 7.6 (dt, 1H), 8.45 (m, 2H). Anal. ($C_{23}H_{28}N_4O_2$) C, H, N.

5*E*-6-(3-(2-Carbamoyl-3-(2-methylpropyl)guanidino)-phenyl)-6-(3-pyridyl)hex-5-enoic acid (45). Compound **30** (1.34 g, 3.3 mmol) was stirred in 4 M HCl (50 mL) for 48 h at 25 °C. The solution was buffered to pH 5−6 by addition of NaOAc and extracted with EtOAc (3×). The organic phase was concentrated, and the residue was purified by column chromatography on silica gel with CH_2Cl_2/EtOH 19:1 to yield 0.76 g (54%) of **45** as a foam: ^{1}H NMR ($CDCl_3$) 0.75 (d, 6H), 1.8 (m, 3H), 2.1−2.4 (m, 4H), 2.8 (bs, 2H), 3.35 (q, 2H), 6.2 (t, 1H), 7.0 (d, 1H), 7.1 (m, 2H), 7.2 (m, 1H), 7.4 (dd, 1H), 7.5 (dt, 1H), 8.45 (m, 2H), 8.9 (bs,2H). Anal. ($C_{23}H_{29}N_5O_3$) C, H, N.

5*E*-6-(3-(2-Benzoyl-3-cyclopropyl-guanidino)phenyl)-6-(3-pyridyl)hex-5-enoic Acid (46). Preparation was performed according to **29** starting from **15** and cyclopentylamine. Recrystallization from EtOAc/^{i}PrOH yields **46** in 76%: ^{1}H NMR (DMSO-d_6) 0.9−1.3 (m, 8H), 1.95−2.25 (m, 6H), 4.3 (m, 1H), 6.25 (t, 1H), 6.95 (d, 1H), 7.2−7.65 (m, 8H), 7.95 (d, 2H), 8.45 (m, 2H), 10.75 (bs, 1H), 12.0 (bs, 1H). Anal. ($C_{30}H_{32}N_4O_3$) C, H, N.

5*E*-6-(3-(3-*tert*-Butyl-2-methylsulfonyl-guanidino)-phenyl)-6-(3-pyridyl)hex-5-enoic Acid (47). The preparation was performed according to **29** starting from **20** and *tert*-butylamine. Purification was effected by recrystallization from EtOAc/diisopropyl ether; yield 85%: ^{1}H NMR ($CDCl_3$) 1.35 (s, 9H), 1.85 (q, 2H), 2.2 (q, 2H), 2.35 (t, 2H), 3.05 (s, 3H), 4.8 (s, 1H), 6.15 (t, 1H), 7.0−7.25 (m, 4H), 7.45 (m, 2H), 8.5 (m, 2H), 8.6 (s, 1H). Anal. ($C_{23}H_{30}N_4O_4S$) C, H, N, S.

5*E*-6-(3-(3-*tert*-Butyl-2-phenylsulfonyl-guanidino)-phenyl)-6-(3-pyridyl)hex-5-enoic Acid (48). The preparation was performed according to **29** starting from **21** and *tert*-butylamine. Purification was effected by column chromatography with CH_2Cl_2/EtOH 30:1; yield 73%: ^{1}H NMR ($CDCl_3$) 1.3 (s, 9H), 1.85 (q, 2H), 2.2 (q, 2H), 2.35 (t,2H), 4.85 (bs, 1H), 6.15 (t, 1H), 6.9 (s, 1H), 7.1 (m, 2H), 7.2 (m, 1H), 7.35−7.5 (m, 5H), 7.95 (m, 2H), 8.5 (bs, 2H), 8.85 (bs, 1H); Anal. ($C_{28}H_{32}N_4O_4S$) C, H, N, S.

5*E*-6-(3-(2-(3-(2-Methylpropyl)-2-(tetrazol-5-yl)guanidino)phenyl)-6-(3-pyridyl)-hex-5-enoic Acid (49). Compound **30** (1.72 g, 4.2 mmol) and tributyltin azide (2.13 g, 6.4 mmol) were refluxed in toluene (50 mL) for 4 h. The reaction mixture was concentrated and the residue was purified by column chromatography with CH_2Cl_2/EtOH 19:1 + 2% acetic acid and by subsequent recrystallization from EtOAc to yield 1.21 g (64%) of **49**: ^{1}H NMR (DMSO-d_6) 0.95 (d, 6H), 1.7 (q, 2H), 1.9 (m, 1H), 2.2 (m, 4H), 3.3 (t, 2H), 6.2 (t, 1H), 6.9 (d, 1H), 7.1 (s, 1H), 7.3−7.45 (m, 2H), 7.6 (m, 2H), 8.3 (bs, 2H), 8.45 (m, 2H), 8.9 (bs, 1H). Anal. ($C_{23}H_{28}N_8O_2$) C, H, N.

5*E*-6-(3-(1-Cyclohexylamino-2-nitro-ethylenamino)-phenyl)-6-(3-pyridyl)hex-5-enoic Acid (50). The preparation was performed according to **29** starting from **17** and cyclohexylamine. Purification was effected by recrystallization from EtOAc/^{i}PrOH; yield 55%: ^{1}H NMR (DMSO-d_6) 1.1−1.5 (m, 5H), 1.5−1.8 (m, 5H), 1.9 (m, 2H), 2.05−2.3 (m, 4H), 3.75 (m, 1H), 6.2 (s, 1H), 6.25 (t, 1H), 7.0 (s, 1H), 7.1 (d, 1H), 7.2−7.4 (m, 2H), 7.45−7.6 (m, 2H), 8.45 (m, 2H), 10.1 (bs, 1H), 12.0 (bs, 1H). Anal. ($C_{25}H_{30}N_4O_4$) C, H, N.

5*E*-6-(3-(2,2-Dicyano-1-*tert*-butylamino-ethylenamino)-phenyl)-6-(3-pyridyl)hex-5-enoic Acid (51). Compound **23** (2.1 g, 5 mmol) was dissolved in CH_2Cl_2 (100 mL), and mCPBA (50%) (1.9 g, 6 mmol) was added in portions over a period of 1.5 h at 25 °C. *tert*-Butylamine (10 mL, 95 mmol) was added, and the solution was stirred for 12 h at 25 °C. The reaction mixture was washed with water, dried, and concentrated. The saponification of the methyl ester was performed according to **29**. Purification was effected by column chromatography with CH_2Cl_2/EtOH 20:1 followed by recrystallization from EtOAc/diisopropyl ether; yield 43%: ^{1}H NMR (DMSO-d_6) 1.35 (s, 9H), 1.65 (q, 2H), 2.2 (m, 4H), 6.25 (t, 1H), 6.9 (m, 2H), 7.1 (d, 1H), 7.25−7.45 (m, 2H), 7.55 (s, 1H), 7.6 (dt, 1H), 8.45 (m, 2H), 8.7

(s, 1H), 12.0 (s, 1H); IR (KBr) 2205, 2180 (CN) cm^{-1}. Anal. ($C_{25}H_{27}N_5O_2$) C, H, N.

5*E*-6-(3-(4-*tert*-Butyl-2,3-dioxo-4-cyclobut-1-enyl)aminophenyl)-6-(3-pyridyl)hex-5-enoic Acid (52). Compound **25** (3.0 g, 7 mmol) and *tert*-butylamine (2 mL, 19 mmol) in ^{i}PrOH (50 mL) were stirred for 48 h at 25 °C. The solution was concentrated and the residue was chromatographed on silica gel with CH_2Cl_2/EtOH 25:1 to yield 2.38 g of the methyl ester (mp 154−155 °C). The saponification of the methyl ester was performed according to **29**. Purification was effected by column chromatography with CH_2Cl_2/EtOH 15:1 followed by recrystallization from EtOAc/diisopropyl ether, yield 60%: ^{1}H NMR (DMSO-d_6) 1.4 (s, 9H), 1.7 (q, 2H), 2.2 (m, 4H), 6.2 (t, 1H), 6.8 (d, 1H), 7.1 (s, 1H), 7.25−7.45 (dd + t, 2H), 7.55 (m, 2H), 7.85 (s, 1H), 8.45 (m, 2H), 9.8 (s, 1H), 12.0 (s, 1H); IR (KBr) 1790 (C═O) cm^{-1}. Anal. ($C_{25}H_{27}N_3O_4$) C, H, N.

4*E/Z*-5-(3-Acetylaminophenyl)-5-(3-pyridyl)pent-4-enoic Acid (55). The preparation was performed using the same protocol as for compound **12** starting from **54** and 3-carboxypropyl-triphenylphosphonium bromide; yield 74% (viscous oil): ^{1}H NMR ($CDCl_3$ + CD_3OD) 2.15 (s, 3H), 2.4 (m, 4H), 6.15 (dt, 1H), 6.9 (d, 1H), 7.2−7.5 (m, 5H), 8.4 (m, 2H). Anal. ($C_{18}H_{18}N_2O_3$) C, H, N.

6*E*-7-(3-Acetylaminophenyl)-7-(3-pyridyl)hept-6-enoic Acid (56). The preparation was performed using the same protocol as for compound **12** starting from **54** and 5-carboxypentyl-triphenylphosphonium bromide; yield 72% (viscous oil): ^{1}H NMR ($CDCl_3$ + CD_3OD) 1.4−1.7 (m, 4H), 2.15 (s, 3H), 2.4 (m, 4H), 6.15 (t, 1H), 6.85 (d, 1H), 7.2−7.5 (m, 5H), 8.4 (m, 2H). Anal. ($C_{20}H_{22}N_2O_3$) C, H, N.

7*E*-8-(3-Acetylaminophenyl)-8-(3-pyridyl)oct-7-enoic acid (57). The preparation was performed using the same protocol as for compound **12** starting from **54** and 6-carboxyhexyl-triphenylphosphonium bromide; yield 69%, mp 149−151 °C: ^{1}H NMR (DMSO-d_6) 1.2−1.5 (m, 6H), 2.05 (s, 3H), 2.15 (m, 4H), 6.2 (t, 1H), 6.85 (d, 1H), 7.25−7.4 (m, 3H), 7.5−7.6 (m, 2H), 8.45 (m, 2H), 9.9 (s, 1H), 12.0 (s, 1H). Anal. ($C_{21}H_{24}N_2O_3$) C, H, N.

4*E*-5-(3-(2-Cyano-3-*tert*-butyl-guanidino)phenyl)-5-(3-pyridyl)pent-4-enoic Acid (58). Compound **55** (4.5 g, 14.5 mmol) was stirred in MeOH (150 mL) and HCl in MeOH (7M, 20 mL) for 18 h at 25 °C. The mixture was concentrated, and the residue was taken up in water, adjusted to pH 8 by addition of $NaHCO_3$, and extracted with EtOAc. The organic was evaporated, and the residue was dissolved in ^{i}PrOH. **9** (4.3 g, 18 mmol) was added and the mixture was stirred for 18 h at 25 °C, filtered, and concentrated. This raw material was then reacted with *tert*-butylamine (3 mL, 26 mmol) according to the protocol described for compound **29**. Purification was effected by column chromatography with CH_2Cl_2/EtOH 20:1 and by subsequent recrystallization from EtOAc/^{i}PrOH; yield 0.48 g (17%): ^{1}H NMR (DMSO-d_6) 1.3 (s, 9H), 2.35 (m, 4H), 6.2 (t, 1H), 6.8−6.95 (m, 2H), 7.15 (d, 1H), 7.3−7.45 (t + dd, 2H), 7.6 (dt, 1H), 8.4 (m, 2H), 9.05 (s, 1H), 12.1 (bs, 1H); IR (KBr) 2180 (CN) cm^{-1}. Anal. ($C_{22}H_{25}N_5O_2$) C, H, N.

6*E*-7-(3-(2-Cyano-3-*tert*-butyl-guanidino)phenyl)-7-(3-pyridyl)hept-6-enoic Acid (59). The preparation was performed using the same protocol as for compound **58** starting from **56**. Purification was effected by column chromatography with CH_2Cl_2/EtOH 15:1 and by subsequent recrystallization from EtOAc/diisopropyl ether; yield 30%: ^{1}H NMR ($CDCl_3$) 1.4 (s, 9H), 1.6 (m, 4H), 2.1 (m, 2H), 2.35 (m, 2H), 5.1 (s, 1H), 6.2 (t, 1H), 6.95 (d, 1H), 7.2 (m, 3H), 7.4 (t, 1H), 7.55 (dt, 1H), 8.45 (m, 2H), 8.8 (s, 1H); IR (KBr) 2160 (CN) cm^{-1}. Anal. ($C_{24}H_{29}N_5O_2$) C, H, N.

7*E*-8-(3-(2-Cyano-3-*tert*-butyl-guanidino)phenyl)-8-(3-pyridyl)oct-7-enoic Acid (60). The preparation was performed using the same protocol as for compound **58** starting from **57**. Purification was effected by column chromatography with CH_2Cl_2/EtOH 15:1 and by subsequent recrystallization from EtOAc; yield 29%: ^{1}H NMR ($CDCl_3$) 1.4 (s + m, 11H), 1.55 (m, 4H), 2.1 (m, 2H), 2.35 (m, 2H), 5.05 (s, 1H), 6.2 (t, 1H), 6.95 (d, 1H), 7.2 (m, 3H), 7.4 (t, 1H), 7.55 (dt, 1H), 8.45 (m, 2H), 8.6 (s, 1H); IR (KBr) 2160 (CN) cm^{-1}. Anal. ($C_{25}H_{31}N_5O_2$) C, H, N.

(3-Nitrophenyl)-(3-pyridyl)-ketoxime (62). Compound **61** (26.5 g, 116 mmol) and NH_2OH·HCl (10.42 g, 115 mmol) were refluxed for 2 h in a mixture of EtOH (200 mL) and pyridine (50 mL). The reaction mixture was evaporated, and the residue was suspended in EtOH/water and adjusted to pH 4−5 by addition of formic acid. The precipitate was filtered with suction, washed with water, and dried at 60 °C to yield 24.6 g (87%) of **62** as an *E*/*Z*-mixture (1:1): ^{1}H NMR (DMSO-d_6) 7.45 + 7.55 (m, 1H), 7.65−7.85 (m, 3H), 8.2−8.4 (m, 2H), 8.55−8.7 (m, 2H), 12.05 (s, 1H); IR (KBr) 1350, 1530 (NO_2) cm^{-1}. Anal. ($C_{12}H_9N_3O_3$) C, H, N.

***O*-(4-Ethoxycarbonylbutyl)-(3-nitrophenyl)-(3-pyridyl)-ketoxime (63).** Compound **62** (24.7 g, 100 mmol) was dissolved in DMF (200 mL), and KOtBu (12.6 g, 110 mmol) was added followed by ethyl 5-bromopentanoate (22.4 g, 107 mmol). The mixture was stirred for 0.5 h at 25 °C. The solvent was evaporated, and the residue was taken up in water and extracted with CH_2Cl_2. The organic phase was concentrated, and the residue was purified by column chromatography with cyclohexane/EtOAc 1:1 to yield 31.9 g (85%) of **63** (viscous oil) as an *E*/*Z*-mixture: ^{1}H NMR ($CDCl_3$) 1.25 (t, 3H), 1.7 (m, 4H), 2.3 (m, 2H), 4.1 (q, 2H), 4.25 (m, 2H), 7.3 + 7.45 (m, 1H), 7.5−7.8 (m, 3H), 8.2−8.4 (m, 2H), 8.6−8.75 (m, 2H); IR (KBr) 1730 (C═O), 1350, 1530 (NO_2) cm^{-1}. Anal. ($C_{19}H_{21}N_3O_5$) C, H, N.

***E*-*O*-(4-Ethoxycarbonylbutyl)-(3-(2-cyano-3-*tert*-butyl-guanidino)phenyl)-(3-pyridyl)ketoxime (64) and *Z*-*O*-(4-Ethoxycarbonylbutyl)-(3-(2-cyano-3-*tert*-butyl-guanidino)-phenyl)-(3-pyridyl)ketoxime (65).** Compound **63** (18 g, 48 mmol) was hydrogenated at 25 °C in EtOH (200 mL) with Raney-Ni (2 g) for 5 h at a pressure of 5 bar. The mixture was filtered, concentrated, and purified by column chromatography with 1,2-dichloroethane/EtOH 19:1 to yield 9 g. The residue was reacted with **9** (6.35 g, 26 mmol) in ^{i}PrOH (130 mL) for 12 h at 25 °C. The reaction mixture was filtered, and the filtrate was concentrated and reacted with *tert*-butylamine (13 mL, 112 mmol) according to the procedure described for compound **29**. Separation of the isomers was performed by column chromatography with CH_2Cl_2/EtOH 30:1. The faster running fraction yields 0.79 g (7.5%) of **64** after recrystallization from EtOAc/diisopropyl ether: ^{1}H NMR (DMSO-d_6) 1.3 (s, 9H), 1.5−1.7 (m, 4H), 2.25 (t, 2H), 4.15 (t, 2H), 6.9 (s, 1H), 7.05 (m, 2H), 7.2 (d, 1H), 7.4 (m, 2H), 7.8 (dt, 1H), 8.6 (m, 2H), 9.05 (s, 1H), 12.0 (s, 1H); IR (CH_2Cl_2) 2160 (CN) cm^{-1}. Anal. ($C_{23}H_{28}N_6O_3$) C, H, N.

The slower running fraction yields 1.2 g (11%) of **65** after recrystallization from EtOAc/diisopropyl ether: ^{1}H NMR (DMSO-d_6) 1.25 (s, 9H), 1.45−1.7 (m, 4H), 2.2 (t, 2H), 4.15 (t, 2H), 6.9 (s, 1H), 7.1−7.2 (m, 3H), 7.35 (t, 1H), 7.5 (dd, 1H), 7.75 (dt, 1H), 8.5 (d, 1H), 8.65 (dd, 1H), 9.1 (s, 1H), 12.0 (s, 1H); IR (KBr) 2180 (CN) cm^{-1}. Anal. ($C_{23}H_{28}N_6O_3$) C, H, N.

(b) X-ray analysis. Compound **32** exists in form of a polycrystalline powder and had to be recrystallized. After preliminary solubility tests, attempts were made to carry out crystallization in various organic solvents or solvent mixtures. By far the best crystals were obtained by slow evaporation (about 2−3 days) from an acetone/water mixture.

Crystallographic data: unit cell parameters a = 17.058(9) Å, α = 90.0(−)°, b = 19.713(4) Å, β = 109.79(4)°, c = 15.446(6) Å, γ = 90.0(−)°; formula $C_{23}H_{27}N_5O_2$·0.5CH_3COCH_3; formula weight 434.54 g/mol; formula units/cell 8; calculated density 1.181 g/cm^3; space group monoclinic *C*2/*c*; radiation wavelength 1.5418 Å (Cu Kα, Ni-filtered); number of independent reflections 3989; number of unobserved reflections (I < 26) 281; final R value 5.9%. Method of data collection: STOE four-circle diffractometer, ω−2Θ scan technique. Method of structure solution: direct methods using SHELXS86 program.[33] Method of refinement: the structure was refined first with isotropic and then with anisotropic temperature factors by the "least squares" method (XTAL[34]) using unit weights. Statement of features on the final Fourier map: no significant features.

(c) Biochemistry. Materials and Methods. SQ 29,548-[5,6-^{3}H(N)] (code NET-936), the 6-keto-$PGF_{1\alpha}$ RIA kit (code

NEK-008), cAMP-[2,8-^{3}H] (code NET-275), and sucrose-[^{14}C-(U)] (code NEC-100) were purchased from New England Nuclear (Dreieich, Germany). U 46,619 was purchased from Sigma and Sepharose 2B from Pharmacia. Collagen and SKF dilution buffer were obtained from Hormon-Chemie or Nycomed (Munich, FRG). All other chemicals were of the highest purity commercially available. All experiments were performed at least in duplicate.

Thromboxane receptor binding, thromboxane synthase inhibition, collagen-induced aggregation in human platelet-rich plasma, and whole blood- as well as collagen-induced prostacyclin production were determined according to ref 23.

(d) Pharmacology. Inhibition of Recurrent Thrombus Formation in Anesthetized Rabbits. Twenty rabbits of either sex between 2.9 and 3.7 kg (New Zealand White, Boehringer Ingelheim Pharma KG, Biberach, Germany) were studied while under general anesthesia produced with xylazine hydrochloride and ketamine hydrochloride administered intravenously through an ear vein. The jugular vein was cannulated for the administration of terbogrel. The carotid artery was cannulated with a saline-filled polyethylene cannula through which arterial blood pressure was measured using an external transducer. Through a midline abdominal incision, the abdominal aorta was exposed by retracting the gut. Distal to the branch point of the renal arteries, the abdominal aorta was dissected free and a 2.0 mm diameter Doppler flow probe was implanted around the vessel. Flow velocity through the vessel was measured using a pulsed Doppler flow system (Triton Technologies, San Diego).

Protocol. Upon completion of the surgical preparation and stabilization of the arterial blood pressure, the aorta was compressed distal to the flow probe for 5 min using a hemostat. The resultant clot was dislodged mechanically, and a constrictive device was placed at the site of the vascular injury caused by the clamp. The constrictor was tightened to produce a flow-limiting stenosis to stimulate local thrombus formation. Upon placement of the constrictor, flow through the aorta then decreased spontaneously and progressively to zero. When flow reached zero, the thrombus was dislodged mechanically but without removing or modifying the severity of the stenosis. A new thrombus then followed spontaneously, resulting in reproducible and repetitive occlusive arterial thrombus formation.

A control period of 30 min, in which the reproducibility of the recurrent thrombus formation was established, preceded the administration of terbogrel. Terbogrel was administered as a bolus infusion over 30 s into the jugular vein and flushed with warm isotonic saline solution. A 60 min observation period then followed. Four doses of terbogrel were administered (0.1, 0.3, 1.0, and 3.0 mg/kg) in four independent groups of rabbits. Each group consisted of four rabbits except for the group receiving 0.3 mg/kg, which consisted of eight.

Following the 1 h observation period after the administration of terbogrel, the rabbits were killed with an intravenous injection of 20% KCl solution.

Measurements. The intensity of thrombosis was assessed by measuring the frequency of occlusive thrombus formation. Aortic flow velocity and arterial blood pressure were recorded on a multichannel chart recorder at a speed of 5 mm/min. Arterial blood pressure was monitored to assess the level of anesthesia throughout the study. Although the arterial blood pressure data were not analyzed, there were no marked changes in arterial blood pressure through the study.

Terbogrel was dissolved in (1:10) NaOH and 0.9% NaCl to produce 0.05%, 0.2%, 0.5%, or 2% solutions allowing for similar injection volumes of 0.4−1.0 mL for doses of 0.1, 0.3, 1.0, 3.0 mg/kg.

Data Reduction and Statistics. The assessment of the local thrombosis severity was made through the measurement of the occlusive frequency at the site of stenosis. To make this calculation, the 1 h following the administration of terbogrel was divided into three 20 min periods. For each period, the rapidity of occlusive thrombus formation was calculated as the number of occlusive thrombi per hour. For the calculation of the dose−response relationship, all three postadministration periods were averaged.

A one-way analysis of variance was applied to test for group differences using *SYSTAT* software.[35] Barlett's test was performed to confirm homogeneity of the group variances prior to applying the analysis of variance. When a group difference was detected, post hoc testing to compare individual mean values was performed using Tukey's test for multiple paired comparisons.[36] A *P*-value less than 0.05 was assumed to indicate a significant difference. The plotting of the dose−response data and calculation of the value for a 50% effective dose (ED_{50} or X_{50}) was done using Biosoft Fig-P software (Cambridge, United Kingdom).

Acknowledgment. We thank Dr. Klaus Wagner for the assignment of the geometrical isomers by ^{1}H and ^{13}C NMR spectroscopy and Prof. Axel Prox and his staff for the analytical and spectral data. We are grateful to Norbert Birk, Ludwig Gutschera, and Ulrich Müller for their skillful technical assistance.

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JM9707941