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Design and Synthesis of an Orally Active GPIIb/IIIa Antagonist Based on a Phenylpiperazine Scaffold

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abstract: The design and synthesis of an orally active LMW non-peptide GPIIb/IIIa antagonist, based on a *N,N'*-bisphenylpiperazine scaffold, is described. The optimal compound showed a high *in vitro* binding potency (pIC_{50}=8.7) in combination with potent oral antithrombotic activity (30-40% inhibition of thrombus growth at 0.3-3 mg/kg) with a duration of action of >90 min. in a hamster cheek pouch model.

Keywords: antagonists, anticoagulants, isosteres, molecular modelling/mechanics.

After activation by thrombin, ADP or collagen the blood platelet GPIIb/IIIa receptors gain a high affinity for fibrinogen (also fibronectin, vitronectin and the von Willebrand factor). Because one fibrinogen dimer has six GPIIb/IIIa binding sites crosslinking occurs leading to thrombus formation. Antagonizing the GPIIb/IIIa receptor may thus inhibit this specific pathway of platelet aggregration. GPIIb/IIIa antagonists may be useful drugs in the prevention of thromboembolic disorders such as acute myocardial infarction, unstable angina and stroke. Therefore, in concurrent cardiovascular drug research, GPIIb/IIIa antagonists are intensively studied.[1]

Literature data reveal that the Arg-Gly-Asp (RGD) tripeptide sequence in fibrinogen is mainly responsible for the binding process with GPIIb/IIIa receptor. The side chain aspartic acid carboxylate group and the strongly basic arginine guanidinium group are the main pharmacophoric elements in the RGD tripeptide. A SAR study with conformationally constrained RGD peptide analogs unveiled the range for the optimal distance between the carbon atoms of the carboxylate and guanidinium functionalities to be in the range of 12.5-16.5 Å.[2]

In this paper we describe our peptidomimetic approach in which the required acidic and basic parts are attached at both ends of the relatively rigid and pharmacologically versatile *N,N*-diphenylpiperazine or 1-phenyl-4-benzylpiperazine spacer molecules (table 1). To replace the originally present basic arginine guanidinium group the bioisosteric *p*-amidinophenyl moiety was selected.[3] The terminal phenyl groups in both benzylpiperazine series were functionalized with *m,p*-carboxymethyl and *m*-carboxymethyleneoxy groups to give **1-6**. In the bisphenylpiperazine series analogs with *m,p*-carboxymethylene (**8,9**), *m,p*-carboxymethyleneoxy (**7,10**), *p*-(2-carboxyethylene) (**11**), *p*-([2-oxy-(2-methyl)propionic acid]) (**12**) and *p*-(4-carboxypropylene) (**13**) groups were prepared. These substitution patterns allow good adjustment of the optimal distance between the main basic and acidic pharmacophores, ranging from 12.4-18.8Å (*vide infra*).

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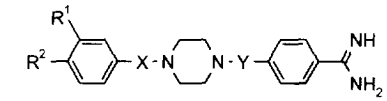
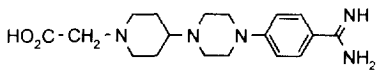
Table 1



1-13



GR144053F

Ro 43-8857

	R^1	R^2	X	Y
1	HO_2C-CH_2-O-	H	-CH_2-	-
2	HO_2C-CH_2-	H	-CH_2-	-
3	H	HO_2C-CH_2-	-CH_2-	-
4	HO_2C-CH_2-O-	H	-	-CH_2-
5	HO_2C-CH_2-	H	-	-CH_2-
6	H	HO_2C-CH_2-	-	-CH_2-
7	HO_2C-CH_2-O-	H	-	-
8	HO_2C-CH_2-	H	-	-
9	H	HO_2C-CH_2-	-	-
10	H	HO_2C-CH_2-O-	-	-
11	H	HO_2C-CH_2-CH_2-	-	-
12	H	HO_2C-$C(CH_3)_2$-O-	-	-
13	H	HO_2C-$(CH_2)_3$-O-		

Also a difference in the spatial positioning of the phenylpiperazine scaffolding unit relative to the terminal pharmacophores is introduced in this way. During the course of our study GR144053F (table 1), which also employs a phenylpiperazine moiety, was published.[4] GR144053F and Ro 43-8857[5] (table 1) have been included in our study as potent reference compounds.

Chemistry

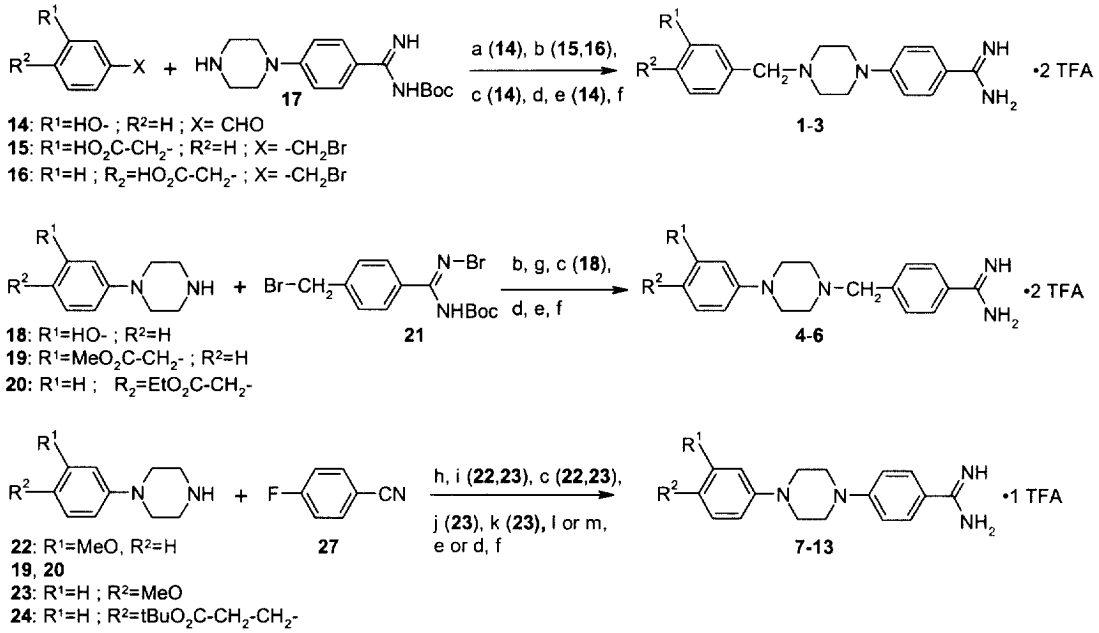
Synthetically compounds **1-13** may be divided into three classes; 1-(*p*-amidino)phenyl-4-benzylpiperazines **1-3**, 1-phenyl-4-(*p*-amidino)benzylpiperazines **4-6** and 1-(*p*-amidino)phenyl-4-phenylpiperazines **7-13**.

Compounds **1-3** were synthesized by functionalization of key building block **17**[6] with the benzaldehyde **14** or the benzylic bromides **15,16**. Due to solubility reasons hydrolysis of the methylester precursor for **1** was conducted under acidic conditions. Removal the amidino Boc protective group by the standard TFA/CH_2Cl_2 procedure yielded the crude compounds **1-3**. Purification by preparative reverse phase HPLC gave **1-3** as di-TFA salts.

Compounds **4-6** were prepared by alkylation of benzylic bromide **21** with the appropriately substituted phenylpiperazines **18-20**.[7] The synthesis of **21** deserves some special attention. NBS mediated bromination of *p-N*-Boc-amidinotoluene occurs preferably at the amidine imine nitrogen atom. Bromination at the benzylic carbon atom only occurs after addition of a second equivalent NBS. Nucleophilic substitution of the benzylic bromide by the piperazine nitrogen atoms proceeded in high yields. Removal of the imine bromine atom could be accomplished easily by extraction with an aqueous $Na_2S_2O_3$ solution.

The main step in the synthesis of the 1,4-diphenylpiperazines **7-13** is the nucleophilic aromatic substitution reaction of fluorine in *p*-fluorobenzonitrile by the functionalized phenylpiperazines **19,20** and **22**-24.[2,4]

Scheme 1 Synthesis



(a) $Ti(OiPr)_4$, $NaCNBH_3$; (b) Et_3N, DMF; (c) $Cl\text{-}CH_2\text{-}CO_2Me$, K_2CO_3, KI, DMF; (d) TFA/CH_2Cl_2=1/1; (e) $HOAc/H_2O$=1/4, reflux; (f) RP-18 preparative HPLC ($MeCN/H_2O$/0.1% TFA); (g) 5% $Na_2S_2O_3$, reflux; (h) K_2CO_3, NMP, reflux; (i) BBr_3, CH_2Cl_2, -75°C; (j) $Br\text{-}(CH_3)_2C\text{-}CO_2tBu$, K_2CO_3, DMF; (k) $Br\text{-}(CH_2)_3\text{-}CO_2tBu$, KI, K_2CO_3, DMF; (l) *i*: H_2S, Et_3N, Pyridine. *ii*: MeI, acetone, reflux. *iii*) NH_4OAc, MeOH, reflux; (m) *i*: $NH_2OH.HCl$, KOtBu, MeOH. *ii*: H_2/Pd(C), HOAc.

Transformation of the nitrile into the amidine was conducted using two methods: in the case of the methyl or ethyl ester containing precursors of **7-10** the commonly applicated three step procedure $CN \rightarrow C(SMe)=NH \rightarrow C(^{+}SMe_2)=NH \rightarrow C(NH_2)=NH$ was followed.[8] The precursors for the amidines **11-13** were treated with hydroxylamine and potassium-*tert*-butoxide followed by catalytic hydrogenation.[4] All final compounds were purified by preparative HPLC and were characterized by NMR and high resolution mass spectroscopy.[9] The purity was determined by quantitative HR-NMR analysis.

Biological Evaluation and Discussion

The potency of compounds **1-13** to inhibit binding of fibrinogen to platelet glycoprotein IIb/IIIa receptors was assessed by an enzyme-linked immunosorbent assay.[10] As reference compounds GR144053F and Ro 43-8857 were included in the tests. The *in vitro* binding data are summarized in table 2. Compound **10**, based on the bisphenylpiperazine scaffold, displays a very high potency (pIC_{50}=8.7), comparable with the included reference compounds GR144053F and Ro 43-8857. In the *N*-phenyl-*N*-benzylpiperazine series only **3** showed a modest potency (pIC_{50}=6.7). Substitution of the carboxymethyleneoxy group in **10** into a carboxyethyl moiety to give **11** gave a 10-fold lower activity in the binding assay. A 1000-fold decrease of activity was obtained by substitution of the carboxymethyleneoxy group in **10** by a carboxyisopropylideneoxy group as in **12**. The measured binding affinities of the reference compounds were in close agreement with their activities as published.[4,5]

Table 2 *In vitro* GPIIb/IIIa binding affinities and molecular modeling data of **1-13** and 2 reference compounds

	pIC_{50} (obs.)	distance (HO_2C-C(=NH)NH_2) (Å)	ΔV	pIC_{50} (calc.)		pIC_{50} (obs.)	distance (HO_2C-C(=NH)NH_2) (Å)	ΔV	pIC_{50} (calc.)
1	5.4	14.5	6.0	5.5	**9**	6.0	14.9	3.9	6.7
2	5.9	14.2	5.6	5.6	**10**	8.7	16.4	0.0	8.6
3	6.7	15.3	5.2	6.0	**11**	7.6	16.3	3.0	7.0
4	4.8	13.0	5.2	5.1	**12**	6.0	16.3	3.9	6.5
5	4.4	12.4	5.5	4.5	**13**	<5.0[a]	18.8	4.2	4.6
6	5.6	14.6	5.1	6.0	**GR144053F**	8.1	14.8		
7	5.7	14.3	5.2	5.8	**Ro 43-8857**	9.0	15.0		
8	5.8	13.0	5.1	5.2					

[a] In the regression analysis a value of 4.5 was used (*vide infra*).

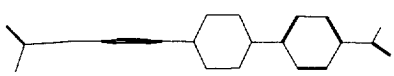
To understand these GPIIb/IIIa *in vitro* binding data **1-13** were subjected to molecular modelling analysis. **1-13** were minimized using the MOPAC/PM3 module of the SYBYL package, version 6.3 (Tripos Associates, Inc., St. Louis, USA), running on a Silicon Graphics Indigo2, Impact 10000.

As can be seen in chart 1 the electron poor *p*-amidino phenyl ring in **10** is coplanar with the piperazine moiety, whereas the other aromatic system is perpendicular to it.[11]

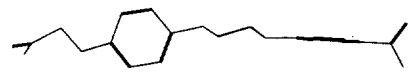
The difference in spatial conformation of the carboxymethyleneoxy group in **10** and a carboxyethyl group in **11** is shown in chart 1. The lone pairs of the arylether oxygen atom in **10** maintains the carboxylic substituent in the plane of the aromatic ring, to give a linear molecule. As can be seen in chart 1 introduction of α,α-dimethyl groups as in **12** gives a non-linear carboxilic acid side chain most probably causing its low binding affinity.

For a QSAR analysis the distance between the carbon atoms of the carboxylate and amidino groups (dis), as well as the volume difference (ΔV) between the most active compound **10** and the other compounds (excluding the reference compounds) mentioned in table 2, have been considered.

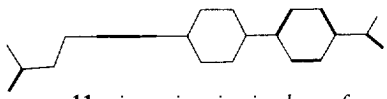
Chart 1 Molecular modelling



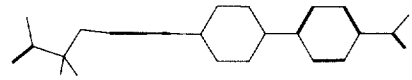
10; *piperazine ring in plane of paper*



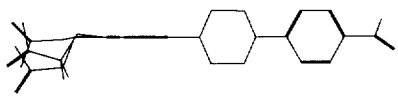
10; *piperazine ring out of plane of paper*



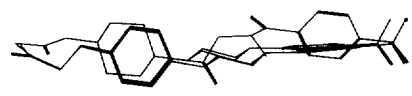
11; *piperazine ring in plane of paper*



12; *piperazine ring in plane of paper*



*overlays **10-12**; piperazine in plane of paper*



*overlays **10**, **GR144053F** and **Ro 43-8857***

For this purpose the compounds were superimposed using the two main pharmacophoric functional groups and the aromatic centroids as fit points. The difference in volume was calculated with the MVOLUME option in SYBYL and is given as the cube root of the original value in order to bring it down to the same dimension as the distance. The calculated data are shown in table 2.

A multiple regression analysis of the observed pIC_{50} values and the calculated dis and ΔV data gives the following equation:

$$pIC_{50}(calc) = -0.54\Delta vol + 4.93dis - 0.16[dis]^2 - 28.84$$
$$(4.8) \quad (4.0) \quad (4.0)$$
$$n=13 \quad r=0.930 \quad F(3.9)=19.2 \quad s=0.50$$

The values between brackets represent the Student t-value.

The optimum distance proved to be 15.4Å and as the volume distance difference between **10** and the other compounds increases, the pIC_{50}(calc) decreases. Although compound **3** exactly fits the optimal distance between the main pharmacophores its non-linear shape prevents high affinity binding at the receptor.

From **10** and the reference compounds *in vitro* functional data were collected by determination of the inhibition of ADP induced platelet aggregration in both guinea-pig and human platelet-rich plasma (table 3).[12]

Table 3 *In vitro* platelet aggregration inhibition

	guinea pig pIC_{50}	human pIC_{50}
10	5.5	6.0
GR144053F	6.6	6.2
Ro 43-8857	6.6	*nd*

Although **10** has a 10-fold lower potency compared to the reference compounds in guinea pig platelet-rich plasma, in human plasma the activities are comparable.

Finally, oral antithrombotic activity of **10** and GR144053 was assessed on ADP induced thrombus formation in the microcirculation of the hamster cheek pouch.[13] For both compounds oral administration of 0.3 mg/kg and 3 mg/kg resulted in 30% and 40% reduction in thrombus growth, respectively, compared to untreated animals.

In conclusion, the pharmacologically versatile phenylpiperazine scaffold was successfully applied to give an orally active GPIIb/IIIa antagonist with nanomolar binding affinity. QSAR analysis revealed the optimum distance between the basic and acidic pharmacophores to be in the 15-16Å range. Also, for obtaining high affinity receptor binding in this series the two pharmacophores must be separated by a linear spacer moiety.

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6 The synthesis of **17** started with piperazination of *p*-aminobenzonitrile with *bis*-2-chloroethylamine hydrochloride in refluxing chlorobenezene for 14h followed by Z protection of the resulting secondary amine. Transformation of the cyano group into an amidine group was accomplished with the three step $CN \rightarrow C(SMe)=NH \rightarrow C(^{+}SMe_2)=NH \rightarrow C(NH_2)=NH$ method. After protection of the amidine moiety with a Boc group and catalytic removal of the Z protective group **17** was obtained.

7 The phenylpiperazines **19-20** were synthesized by heating of methyl-*m*-aminophenylacetate and ethyl-*p*-amino phenylacetate, respectively, with bis-2-chloroethylamine hydrochloride in chlorobenzene at reflux for 14h. The phenylpiperazines **18**, **22** and **23** are commercially available. **24** was obtained after heating of 3-(4-aminophenyl)-propionic acid *tert*-butyl ester (obtained after catalytic hydrogenation of E-3-(4-nitrophenyl)-propenoic acid *tert*-butyl ester) with bis-2-chloroethylamine hydrochloride in chlorobenzene at reflux for 14h.

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9 Spectroscopic data for **10**•TFA: ^{1}H NMR (400 MHz; DMSO-d^6) δ 8.92 (s, 2H, NH_2), 8.80 (s, 2H, NH_2), 7.78 (d, 2H, J=9.0 Hz, PhH_2), 7.11 (d, 2H, J=9.0 Hz, PhH_2), 6.98 (d, 2H, J=9.0 Hz, PhH_2), 6.85 (d, 2H, J=9.0 Hz, PhH_2), 4.57 (s, 2H, OCH_2), 3.55 (m, 4H, $2xNCH_2$), 3.20 (m, 4H, $2xNCH_2$); ^{13}C NMR (100 MHz; DMSO-d^6) δ 170.3 (C=O), 164.3 (H_2N^{+}=C-NH_2), 154.2 (ArC-O), 152.0 (PhC-N), 145.2 (PhC-N), 129.6 (2xPhC), 117.8 (2xPhC), 115.1 ((H_2N^{+}=C-NH_2)-C), 115.0 (2xPhC), 113.4 (2xPhC), 65.0 (O-CH_2), 49.6 ($2xNCH_2$), 46.6 ($2xNCH_2$); CIMS (70eV) exact mass calcd for $C_{19}H_{22}N_4O_3$ m/z, 354,4031 ($[M]^{+}$), found: 354,4028.

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