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Spirocyclic Nonpeptide Glycoprotein IIb–IIIa Antagonists. Part 2: Design of Potent Antagonists Containing the 3-Azaspiro[5.5]undec-9-yl Template

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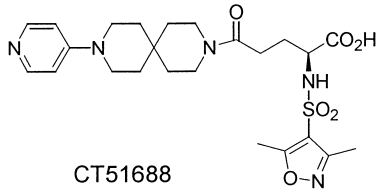
Abstract—The synthesis and biological activity of novel glycoprotein IIb–IIIa anatagonists containing 3-azaspiro[5.5]undec-9-yl nucleus are described. The potent activity of these compounds as platelet aggregation inhibitors demonstrates the utility of the monoazaspirocyclic structure as central template for nonpeptide RGD mimics.

Efforts to discover orally active GPIIb–IIIa antagonists with appropriate pharmaceutical properties for treating thrombotic disease have been numerous.[1] We recently identified the 3,9-diazaspiro[5.5]undecane template as a suitable conformationally constrained nucleus for the preparation of potent and specific GPIIb–IIIa antagonists such as CT51688. (See ref 2: Part 1, preceding paper.) Based on these results we initiated efforts to explore the potential utility of the monoazaspirocyclic template to identify potent GPIIb–IIIa antagonists which would afford more flexibility in appending basic and acidic pharmacophore elements.



CT51688

To begin the investigation of this nucleus, we prepared the ethyl 9-azaspiro[5.5]undecane-3-carboxylate template as described in the literature.[3] The amide-linked and direct-linked benzamidine moieties were incorporated as shown in Schemes 1 and 2. The direct-linked benzamidine series (Scheme 2) was prepared using palladium-catalyzed coupling reactions. A third series of compounds was prepared with direct linkage of the piperidine nitrogen to pyridines, and we again used Pd-catalysis for the synthesis (Schemes 3 and 4).[2]

Also prepared were compounds in which ether- or amine-containing linkages were utilized. (Scheme 4). Finally, a series in which the basic pharmacophore groups were attached through the carboxyl group of the 9-azaspiro[5.5]undecane-3-carboxylate template were prepared as shown in Scheme 5. The cyano group was converted to the corresponding amidines via hydroxylamine addition followed by hydrogenolysis.

The synthesis of compounds **15**–**17** was accomplished by coupling the spironucleus with various α- and β-substituted carboxylic acid chains (Scheme 3). Synthesis of the pyridyl-9-oxa-3-aza-spiro[5.5]undecane nucleus was achieved by spiroannulation methodology, using 4-formylpiperidine and methyl vinylketone under basic conditions (Scheme 4). The carboxyl group of the 9-azaspiro[5.5]undecane template was coupled to the basic bipiperidyl and pyridylpiperazine groups followed by acylation or alkylation of the piperidine ring with acid chloride esters or alkyl bromoesters, respectively (Scheme 5).

All of the synthesized spirocyclic analogues were assayed for their ability to inhibit the aggregation of ADP-stimulated human platelets and to block the

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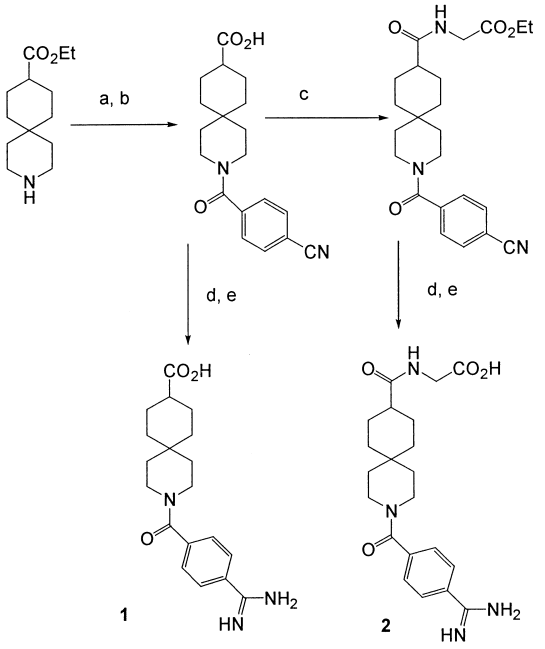
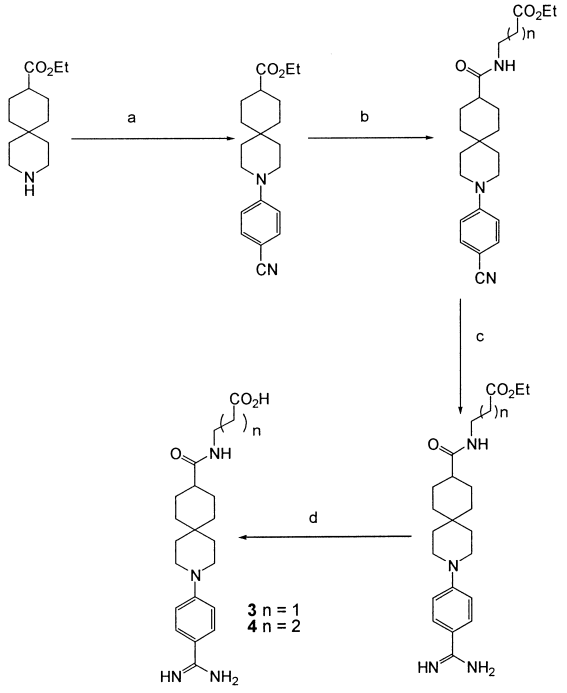
binding of fibrinogen to purified human GPIIb–IIIa.[2] Although only two analogues of the amide-linked benzamidine series were prepared, both displayed weak inhibitory activity (Table 1). Replacement of the amide linkage with the direct-linked benzamidine (analogues **3** and **4**, Table 2) led to enhancement in potency of approximately 7-fold in the platelet aggregation assay and a 160-fold in the fibrinogen binding assay (**3** vs **2**). Compound **3** was significantly more potent in the binding assay compared to the platelet aggregation assay and this may be due to plasma protein binding by this analogue in the aggregation assay in PRP as has been noted in other GPIIb–IIIa inhibitors.[1,4]

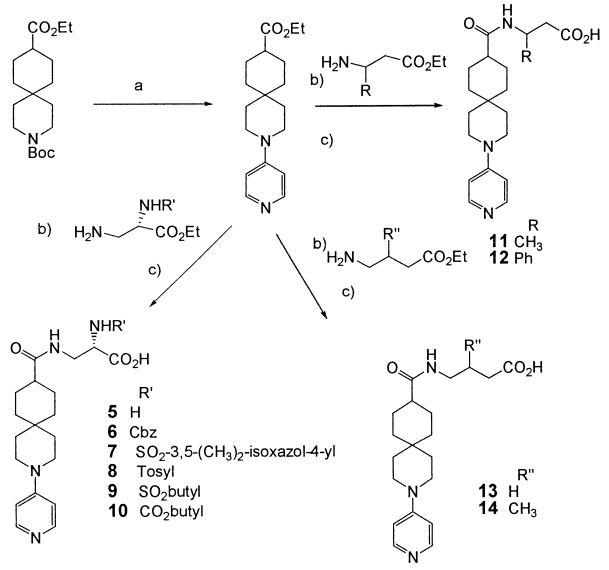


Scheme 1. Synthesis of amide-linked benzamidines **1** and **2**: (a) 2 N HCl, 60 °C; (b) *p*-cyanobenzoyl chloride, Et_3N, DCM; (c) β-alanine ethyl ester, HATU, DIEA, DMF, rt; (d) (i) $NH_2OH{\cdot}HCl$, EtOH, Et_3N, rt; (ii) AcOH, Ac_2O, rt; (iii) 10% Pd/C, H_2, 1 atm; (e) LiOH, THF, H_2O.

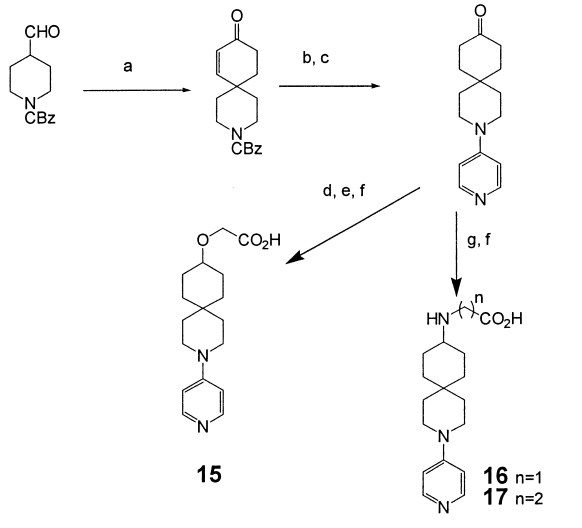


Scheme 2. Synthesis of compounds **3** and **4**: (a) *p*-bromobenzonitrile, *S*-BINAP, NaO*t*Bu, $Pd_2(dba)_3$, toluene, 90 °C, 65%; (b) β-alanine ethyl ester or aminopropionate, HATU, DIEA, DMF, rt, 84%; (c) (i) $NH_2OH{\cdot}HCl$, EtOH, Et_3N, rt; (ii) AcOH, Ac_2O, rt; (iii) 10% Pd/C, H_2, 1 atm; (d) LiOH, THF, H_2O, 63%.

Our next series of analogues utilized the less basic direct linked pyridyl group, a modification chosen to improve both the potency and pharmacokinetic properties. Incorporation of an α-amino substitution at the carboxyl group in the direct linked pyridine series dramatically



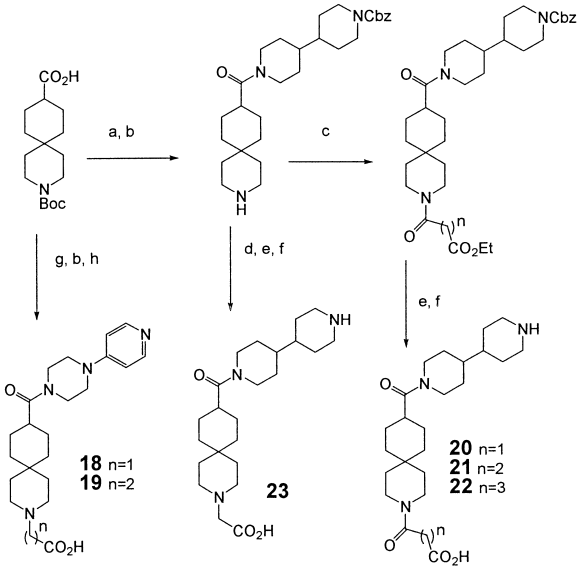
Scheme 3. Synthesis of compounds **5**–**14**: (a) 4-bromopyridine, *S*-BINAP, NaO*t*Bu, $Pd_2(dba)_3$, toluene, 90 °C, 65%; (b) EDC, HOBt, DIEA, DMF, rt, 84%; (c) 3 N HCl, 60 °C, 2 h, 35%.



Scheme 4. Synthesis of pyridyl-9-oxo-3-azaspiro[5.5]undecane analogues: (a) MVK, KOH, EtOH, reflux, 50%; (b) H_2, Pd/C; (c) 4-bromopyridine, *S*-BINAP, NaO*t*Bu, $Pd_2(dba)_3$, toluene, 90 °C, 65%; (d) $NaBH_4$, THF, 65%; (e) ethyl diazoacetate, $[Rh(OAc)_2]_2$, 64%; (f) LiOH, THF, H_2O; (g) β-alanine ethyl ester or aminopropionate, $NaCNBH_3$, AcOH, rt.

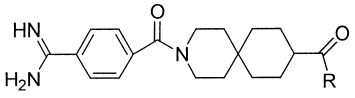
enhances the potency as seen for analogues **6**–**9** (Table 3). Incorporation of the 3,5-dimethylisoxazol-4-yl sulfonamide functionality afforded analogue **7** (CT51819), which displayed 300-fold enhancement in activity in platelet aggregation assay and 830-fold enhancement in binding assay relative to the unsubstituted amine analogue **5** (Table 3). These observations are consistent with the results by a number of other groups and our own observations.[4–6] Of interest, sulfonamide **7** was also found to be a potent inhibitor of ADP-induced platelet aggregation of murine platelets with an IC_{50} value of 45 nM. Thus, this antagonist is unique relative to many potent GPIIb–IIIa antagonists which typically are weak inhibitors of murine GPIIb–IIIa and therefore attractive for study in murine thrombosis models.[7]

The α-carbamate substituted analogue (**10**) resulted in a 10-fold loss in potency relative to butane-sulfonamide analogue (**9**) in the PRP assay and retained similar potency in binding assay (Table 3). There have been extensive SARs at the position β to the carboxylic acid terminus in several different series of GPIIb–IIIa inhibitors.[1,8] It is also reported that activity is greatly



Scheme 5. Synthesis of compounds **18**–**23**: (a) bipiperidyl, BOP, DIEA, DMF, rt; (b) 50% TFA/DCM, 60%; (c) $ClCO(CH_2)_n$-CO_2Et, DCM, DIEA, 92%; (d) $BrCH_2CO_2Et$, K_2CO_3, DMF, 65 °C; (e) LiOH, THF, H_2O; (f) Pd-black, HCO_2NH_4, 85%; (g) pyridyl-piperazine, BOP, DIEA, DMF; (h) $Br(CH_2)_nCO_2Et$, DMF, DIEA, 65 °C, 20%.

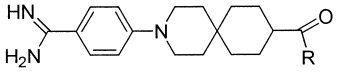
Table 1. In vitro activity for compounds **1** and **2**



Compds	R	ELISA Fg/GPIIb–IIa IC_{50} (μM)[a]	Human PRP IC_{50} (μM)[a]
1	OH	15	19.7
2	$NHCH_2CO_2H$	9.7	38.5

The average error for the determinations was ±15%.
[a]IC_{50} values expressed as the average of at least two determinations.

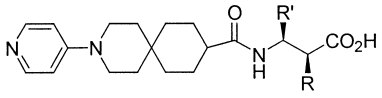
Table 2. In vitro activity for compounds with benzamidine basic unit



Compds	R	ELISA Fg/GPIIb–IIa IC_{50} (μM)[a]	Human PRP IC_{50} (μM)[a]
3	$NH(CH_2)_2CO_2H$	0.060	5.5
4	$NH(CH_2)_3CO_2H$	5	37.1

[a]See Table 1.

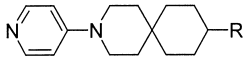
Table 3. Effect of carboxylic acid segment modifications



Analogues	R	R′	ELISA Fg/GPIIb–IIa IC_{50} (μM)[a]	Human PRP IC_{50} (μM)[a]
5	NH_2	H	0.830	12.2
6	NHCBz	H	0.002	2.7
7	$NHSO_2$isoxazole	H	0.001	0.040
8	$NHSO_2$Tos	H	0.001	0.177
9	$NHSO_2$nBut	H	0.003	0.176
10	$NHCO_2$nBut	H	0.008	1.3
11	H	CH_3	0.616	14.3
12	H	Ph	30	77

[a]See Table 1.

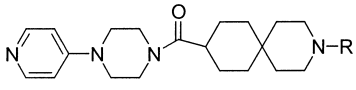
Table 4. In vitro activity for compounds with 4-aminopyridine basic unit



Compds	R	ELISA Fg/GPIIb–IIa IC_{50} (μM)[a]	Human PRP IC_{50} (μM)[a]
13	$CONH(CH_2)_3CO_2H$	0.319	12
14	$CONHCH_2CH(Me)CH_2CO_2H$	0.332	4
15	OCH_2CO_2H	0.25	1.1
16	$NHCH_2CO_2H$	0.16	0.89
17	$NHCH_2CH_2CO_2H$	50	4.5

[a]See Table 1.

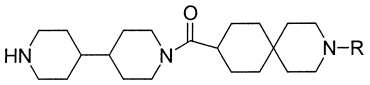
Table 5. In vitro activity for compounds with pyridyl-piperazine basic unit



Compds	R	ELISA Fg/GPIIb–IIa IC_{50} (μM)[a]	Human PRP IC_{50} (μM)[a]
18	CH_2CO_2H	0.364	0.117
19	$CH_2CH_2CO_2H$	3.45	7.9

[a]See Table 1.

Table 6. In vitro activity for compounds with bipiperidyl basic unit



Compds	R	ELISA Fg/GPIIb–IIa IC_{50} (μM)[a]	Human PRP IC_{50} (μM)[a]
20	$COCH_2CO_2H$	2.40	2.5
21	$CO(CH_2)_2CO_2H$	100	50
22	$CO(CH_2)_3CO_2H$	100	50
23	CH_2CO_2H	0.250	0.289

[a]See Table 1.

enhanced by the addition of β-substituents when antagonists contain a β-amino acid functionality.[8] This has been ascribed to favorable interaction with a hydrophobic binding site in GPIIb–IIIa. We have utilized a similar approach to study the effect of β-substitution in our spirocyclic series. The β-phenyl substituted analogue (**12**) resulted in 15-fold loss of potency in PRP and 500-fold in the ELISA assay relative to the unsubstituted analogue **3** (Table 3). The replacement of β-phenyl with the smaller CH_3 residue (**11**) enhanced the activity but overall β-substitution resulted in decreased activity relative to unsubstituted analogue **3** (Table 3). Analogues (**15**–**17**) with ether- or amine-linked carboxylic acid groups did not display enhanced potency, and this approach was not pursued further (Table 4).

In the final series prepared, the basic pharmacophore group was attached to the carboxyl group of the 9-azaspiro[5.5]undecane-3-carboxylate template. We initially focused on optimizing the distance between the carboxylate and basic functionalities. In the pyridylpiperazine series, the extension with a methylene unit (**19**) resulted in 10-fold loss of activity in PRP and fibrinogen binding assay relative to **18** (Table 5). In the bipiperidyl series the amide-linked carboxylic acid analogues **20**–**22** (Table 6) were less potent relative to the alkyl-linked carboxylic acid analogue **23**. The alkyl-linked analogue **23** displayed 10-fold enhancement in activity in both the PRP and binding assay compared to **20**.

We also examined the integrin specificity of all active compounds in this series, and they were found to have IC_{50} values >100 μM against the vitronectin receptor, $\alpha_v\beta_3$. Thus, both direct-linked benzamidine- and pyridine-containing analogues were all highly selective towards GPIIb–IIIa. The pharmacokinetic properties of compounds **7** (CT51819), **8**, **9**, **18**, and **23** as free acids and as their ethyl ester prodrugs were evaluated in Sprague–Dawley rats. The compounds showed low oral bioavailability ($<7\%$) and rapid clearance at 1 mg/kg iv and po dose. Highly active and specific GPIIb–IIIa inhibitors have been discovered in the 6,6-mono-azaspirocyclic series, which extends our previous work with the 6,6-diazaspirocyclic template. However, the poor absorption and pharmacokinetic properties of these inhibitors preclude their ultimate utility as oral GPIIb–IIIa antagonists.

References and Notes

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