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NON-PEPTIDE GLYCOPROTEIN IIb/IIIa INHIBITORS. 6. DESIGN AND SYNTHESIS OF RIGID, CENTRALLY CONSTRAINED NON-PEPTIDE FIBRINOGEN RECEPTOR ANTAGONISTS.

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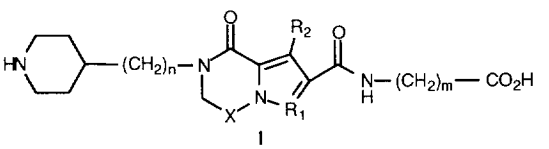
Abstract: Low molecular weight non-peptide inhibitors of platelet aggregation based on rigid bicyclic scaffolds are described. Consideration of the reported conformational preferences of 1-alkyl-3-carbonyl pyrroles led to the synthesis of pyrrolopiperazinone **2a** which was shown to be a potent, selective antagonist.

The activation, adherence, and subsequent aggregation of circulating platelets has been shown to play an important role in various vasoocculsive disorders such as unstable angina, myocardial infarction, transient ischemic attacks, and stroke.[1] The final, common pathway for platelet aggregation, regardless of the activating signal, is the binding of fibrinogen by glycoprotein IIb/IIIa (GPIIb/IIIa) on the surface of activated platelets.[2] This binding is mediated in part by Arg-Gly-Asp (RGD) sequences present in fibrinogen, therefore, small molecules and peptidomemetics that feature elements of this tripeptide sequence have been pursued as inhibitors of platelet aggregation.[3] Several groups have reported compounds that incorporate rigid central constraints that direct the vectors of the pharmacophoric moieties; a guanidine or guanidine surrogate mimicking the arginyl side chain and a carboxylic acid representing the side chain carboxylic acid of aspartic acid.[4-6] A recent report from these laboratories detailed the design, synthesis and pharmocology of a centrally constrained antagonist possessing nanomolar potency.[7]

We now report a series of potent, centrally constrained GPIIb/IIIa inhibitors represented by the generic structure **I**. We selected this framework reasoning that the restricted rotational freedom and reduced peptide character that it affords would result in enhancements in potency and selectivity relative to unconstrained RGD containing tri- and tetrapeptide analogs. This template also allows the effects of structural variation to be rapidly assessed. Modification of the linkers **n** and **m**, reveals both the optimal separation between the piperidine and acid moieties and the optimal position of the rigid central constraint. Variation of $\mathbf{R_1}$ and $\mathbf{R_2}$ allows electronic and steric factors, which influence the conformation of the C-terminal amide, to be probed. Modification of **X** provides a means of varying the flexibility of the aliphatic ring and thus the presentation of the basic side chain. In all of these analogs, piperidine was used as the N-terminus since this group was shown to afford potent analogs that exhibit a high degree of integrin selectivity.[6]

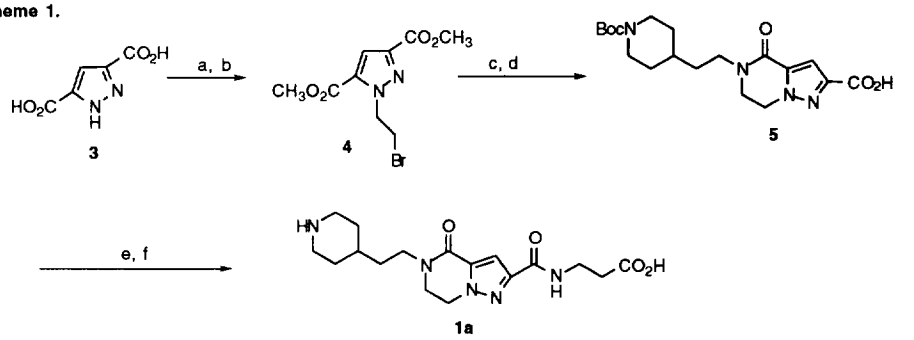


I

Chemistry:

The pyrazolopiperazinone analogs **1a-e** and **1g** (Table 1) were prepared as illustrated for **1a** in Scheme 1. Commercially available 3,5-pyrazoledicarboxylic acid (**3**) was converted to its dimethyl ester and this was alkylated with excess 1,2-dibromoethane to give the bromide **4**. Reaction of 2-(N-Boc-piperidin-4-yl)ethylamine[8] with the bromide **4** and saponification of the resulting ester afforded the pyrazolopiperidinone **5** in 70% yield. Coupling of **5** with β–alanine tert-butyl ester and deprotection of the resulting doubly protected compound with hydrogen chloride gas in ethyl acetate afforded **1a**.

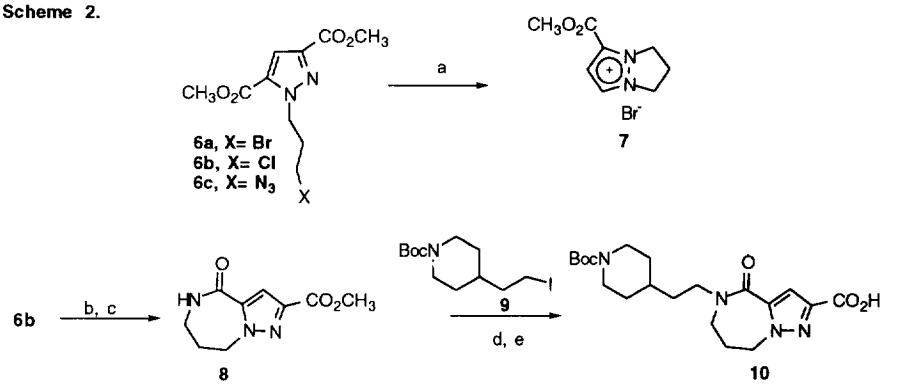
Scheme 1.



(a) HCl, CH_3OH, 65 °C, 100% (b) $BrCH_2CH_2Br$, K_2CO_3, CH_3CN, 80 °C, 97% (c) 2-(N-Boc-piperidin-4-yl)ethylamine, K_2CO_3, CH_3CN, 80 °C, 70% (d) LiOH, THF/H_2O, r.t., 100% (e) $NH_2CH_2CH_2CO_2{}^tBu$ •HCl, Et_3N, EDC, HOBT, DMF, r.t., 95% (f) HCl, EtOAc, 0 °C, 100%.

Analogous preparation of the pyrazolodiazepinone analog **1f** was problematic due to intramolecular cyclization of **6a** to a bicyclic pyrazolium salt which underwent subsequent decarboxylation to afford **7** in high yield (Scheme 2). [9] However, **1f** was prepared by an alternate route as shown in Scheme 2. Treatment of chloride **6b** with excess NaN_3 give the azide **6c** in quantitative yield. The required pyrazolodiazepinone **8** was obtained in 95% yield by treatment of **6c** with palladium on carbon under a hydrogen atmosphere. Alkylation of **8** with **9**, followed by saponification, provided **10**. Conversion of **10** to **1f** was carried out as illustrated in Scheme 1 for the synthesis of **1a**.

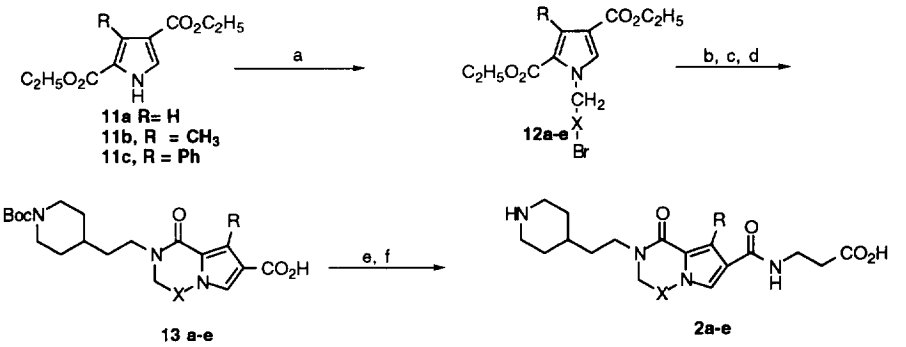
Scheme 2.



(a) 2-(N-Boc-Piperidin-4-yl)ethyl amine, K_2CO_3, CH_3CN, 80 °C, 75% (b) NaN_3, DMSO, r.t., 100% (c) H_2, (30 psi) 10% Pd/C, CH_3OH, 95% (d) NaH, DMF 0 °C to r.t., 86% (e) LiOH, THF/H_2O, r.t., 99%.

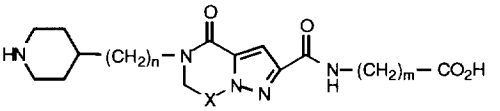
Unlike the pyrazole analogs, both the pyrrolopiperizineones and diazepinones could be prepared by the route shown in Scheme 3. The pyrrole analogs **11a-c** were prepared as previously described.[10]

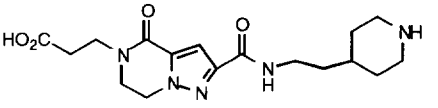
Scheme 3.



(a) NaH, Br-CH_2-X-Br, THF, 65 °C, 73-85% (b) 2-(N-Boc-piperidin-4-yl)ethylamine, K_2CO_3, CH_3CN, 80 °C, 75-88% (c) NaH, THF, 65 °C, 80% (d) LiOH, THF/H_2O, r.t., 98% (e) $NH_2CH_2CH_2CO_2{}^tBu$ •HCl, Et_3N, EDC, HOBT, DMF, r.t., 90-95% (f) HCl, EtOAc, 0 °C, 95%.

Table 1. Pyrazole Based Compounds.

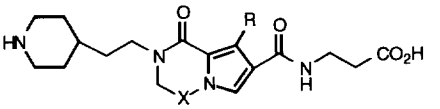


Compound	n	X	m	IC_{50} (μM)[11]
1a	2	-CH_2-	2	0.25
1b	1	-CH_2-	2	45
1c	1	-CH_2-	3	15
1d	3	-CH_2-	1	14
1e	3	-CH_2-	2	1.3
1f	2	-CH_2CH_2-	2	0.47
1g				31

Results and Discussion:

Systematic structural variations of centrally constrained analogs reveals the optimal separation between the acidic and basic groups, the location of the rigid constraint, and the preferred conformation of the acid containing chain. Of the pyrazole based compounds, analog **1a** is the most potent inhibitor of platelet aggregation.[11] Compounds **1c** and **1d**, in which the central constraint is shifted toward either the basic or acidic terminus while maintaining the same overall atom count, demonstrate 60-fold losses in potency. Molecular modeling studies of cyclic peptides, peptide-hybrids, and non-peptides emphasize the requirement of rigorous positioning of the acid side chain.[12,13] Modifications that alter the trajectory of this group have been shown to produce large decreases in potency in non-peptide analogs.[4b] Shortening (**1b**) or lengthening (**1e**) the distance between the termini also leads to reduced activity in comparison to **1a**. Expansion of the piperizinone ring of **1a** to give pyrazolodiazepinone **1f** is associated with a 2-fold loss in activity. Reversal of the orientation of the central constraint (**1g**) gives a >100-fold reduction in potency.

Table 2. Pyrrole Analogs.



Compound	X	R	IC_{50} (μM)
2a	$-CH_2-$	H	0.079
2b	$-CH_2CH_2-$	H	0.17
2c	$-CH_2-$	CH_3	0.17
2d	$-CH_2CH_2-$	CH_3	0.51
2e	$-CH_2CH_2-$	Ph	> 300 μM

Pyrrole based analogs **2a-e** enable the electronic and steric environment of the C-terminal amide moiety to be probed. Solution NMR, molecular modeling, and crystallographic studies of 1-alkyl-3-carbonyl pyrroles demonstrate a preference for conformations in which the carbonyl O and ring N are trans (**14b,** Figure 1).[14,15] This orientation positions the β-alanine in an extended conformation as depicted for the generic structure I. Substitution of pyrrole for the pyrazole of compounds **1a** and **1f** affords analogs **2a** and **2b** (Table 2) which were 3-fold more potent than the analogous pyrazoles suggesting a preference for the extended conformation. Other modifications that influence the environment of the amide carbonyl group include the placement of substituents in the 4-position of the pyrrole ring. Incorporation of groups in this position has been reported to induce out of plane rotations in crystal structures of 3-carbonyl containing analogs.[15] Methylation results in a decrease in activity (**2c** and **d**) while incorporation of a larger phenyl group at this position results in an inactive compound (**2e**). These data also suggest the preference for an extended conformation at the C-terminus.

Figure 1.

14a **14b**

Pyrrolopiperazinone **2a** illustrates the increase in potency that can be realized in an RGD mimetic by using a central constraint to advantageously position the pharmacophoric termini. Despite its lower molecular weight and lack of chirality, (MW = 362, IC_{50} = 0.079 μM), **2a** is >300-fold more potent as an inhibitor of platelet aggregation than the linear tetrapeptide Arg-Gly-Asp-Ser (RGDS, MW = 433, IC_{50} = 26 μM).[6] Moreover, unlike the tetrapeptide RGDS, which has similar affinity for several integrins,[16] **2a** is a selective inhibitor of platelet aggregation. The selectivity of **2a** was evaluated by comparing its ability to inhibit platelet aggregation with its ability to inhibit the binding of human umbilical vein endothelial cells (HUVEC) to fibrinogen, vitronectin and fibronectin coated surfaces.[17] No inhibition of HUVEC binding was observed at 300 μM concentrations **2a**, indicating >3,800-fold selectivity for GPIIb/IIIa.

In summary, novel bicyclic ring systems have been identified as appropriate templates for the construction of potent, selective, low molecular weight inhibitors of platelet aggregation. Structure-activity studies have established features necessary for activity. Consideration of the reported conformational preferences of 1-alkyl-3-carbonyl pyrroles led to the synthesis of pyrrolopiperazinone **2a**, which was more potent than the corresponding pyrazole analog **1a**. Efforts to enhance the potency, and oral activity of these compounds by modulating their physical chemical properties are underway.

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